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# PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)					
Given Name (first and middle [if any] )		Family Name or Surname		Residence (City and either State or Foreign Country)	
Steven		MAH		San Diego, California	
Andreas		BRAUN		San Diego, California	
Stefan M.		KAMMERER		San Diego, California	
Additional inventors are being named on the <u>1</u> separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
METHODS FOR IDENTIFYING RISK OF OSTEOARTHRITIS AND TREATMENTS THEREOF					
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[Page 1 of 2]

Respectfully submitted,

Date April 1, 2004

SIGNATURE

TYPED OR  
PRINTED NAME

Bruce D. Grant

REGISTRATION NO.  
(if appropriate)

47,608

TELEPHONE

(858) 720-7962

Docket Number:

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INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Matthew Roberts	NELSON	San Marcos, California
Rikard Henry	RENELAND	San Diego, California
Maria L.	LANGDOWN	San Diego, California

## METHODS FOR IDENTIFYING RISK OF OSTEOARTHRITIS AND TREATMENTS THEREOF

### Field of the Invention

[0001] The invention relates to genetic methods for identifying risk of osteoarthritis and treatments that specifically target such diseases.

### Background

[0002] Osteoarthritis (OA) is a chronic disease usually affecting weight-bearing synovial joints. There are approximately 20 million Americans affected by OA and it is the leading cause of disability in the United States. In addition to extensive human suffering, OA also accounts for nearly all knee replacements and more than half of all hip replacements in the United States. Despite its prevalence, OA is poorly understood and there are few treatments available besides anti-inflammatory drugs and joint replacement.

[0003] Most commonly affecting middle-aged and older people, OA can range from very mild to very severe. It affects hands and weight-bearing joints such as knees, hips, feet and the back. Knee OA can be as disabling as any cardiovascular disease except stroke.

[0004] OA is characterized by the breakdown of cartilage in joints. Cartilage in joints cushions the ends of bones, and cartilage breakdown causes bones to rub against each other, causing pain and loss of movement. Type II collagen is the main component of cartilage, comprising 15-25% of the wet weight, approximately half the dry weight, and representing 90-95% of the total collagen content in the tissue. It forms fibrils that endow cartilage with tensile strength (Mayne, R. Arthritis Rheum. 32:241-246 (1989)).

### Summary

[0005] It has been discovered that certain polymorphic variations in human genomic DNA are associated with osteoarthritis. In particular, polymorphic variants in loci containing *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* and *ERG* regions in human genomic DNA have been associated with risk of osteoarthritis. The *PSMB1*, *TBP* and *PDCD2* regions are located in a larger region referred to herein as the *chrom 6* region.

[0006] Thus, featured herein are methods for identifying a subject at risk of osteoarthritis and/or a risk of osteoarthritis in a subject, which comprise detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in or around the loci described herein in a human nucleic acid sample. In an embodiment, two or more polymorphic variations are detected in two or more regions, of which one or more is a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* region. In certain

embodiments, 3 or more, or 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more polymorphic variants are detected.

[0007] Also featured are nucleic acids that include one or more polymorphic variations associated with occurrence of osteoarthritis, as well as polypeptides encoded by these nucleic acids. In addition, provided are methods for identifying candidate therapeutic molecules for treating osteoarthritis, as well as methods for treating osteoarthritis in a subject by identifying a subject at risk of osteoarthritis and treating the subject with a suitable prophylactic, treatment or therapeutic molecule.

[0008] Also provided are compositions comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and/or a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid, with a RNAi, siRNA, antisense DNA or RNA, or ribozyme nucleic acid designed from a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence. In an embodiment, the RNAi, siRNA, antisense DNA or RNA, or ribozyme nucleic acid is designed from a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence that includes one or more polymorphic variations associated with osteoarthritis, and in some instances, specifically interacts with such a nucleotide sequence. Further, provided are arrays of nucleic acids bound to a solid surface, in which one or more nucleic acid molecules of the array have a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence, or a fragment or substantially identical nucleic acid thereof, or a complementary nucleic acid of the foregoing. Featured also are compositions comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and/or a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* polypeptide, with an antibody that specifically binds to the polypeptide. In an embodiment, the antibody specifically binds to an epitope in the polypeptide that includes a non-synonymous amino acid modification associated with osteoarthritis (e.g., results in an amino acid substitution in the encoded polypeptide associated with osteoarthritis). In certain embodiments, the antibody selectively binds to an epitope in the *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* polypeptide having an amino acid associated with osteoarthritis.

#### Brief Description of the Drawings

[0009] Figures 1A-1D show proximal SNPs in *chrom 6*, *ELP3*, *CHDC1* and *ERG* regions of genomic DNA, respectively. The position of each SNP in the chromosome is shown on the x-axis and the y-axis provides the negative logarithm of the p-value comparing the estimated allele to that of the control group. Also shown in the figures are exons and introns of the regions in the approximate chromosomal positions.

#### Detailed Description

[0010] It has been discovered that a polymorphic variant in a locus containing a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* region is associated with occurrence of osteoarthritis in subjects. Thus,

detecting genetic determinants associated with an increased risk of osteoarthritis occurrence can lead to early identification of a predisposition to osteoarthritis and early prescription of preventative measures. Also, associating a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* polymorphic variant with osteoarthritis has provided new targets for screening molecules useful in treatments of osteoarthritis.

#### Osteoarthritis and Sample Selection

[0011] Osteoarthritis (OA), or degenerative joint disease, is one of the oldest and most common types of arthritis. It is characterized by the breakdown of the joint's cartilage. Cartilage is the part of the joint that cushions the ends of bones, and its breakdown causes bones to rub against each other, causing pain and loss of movement. Type II collagen is the main component of cartilage, comprising 15-25% of the wet weight, approximately half the dry weight, and representing 90-95% of the total collagen content in the tissue. It forms fibrils that endow cartilage with tensile strength (Mayne, R. Arthritis Rheum. 32:241-246 (1989)).

[0012] Most commonly affecting middle-aged and older people, OA can range from very mild to very severe. It affects hands and weight-bearing joints such as knees, hips, feet and the back. Knee OA, can be as disabling as any cardiovascular disease except stroke.

[0013] Osteoarthritis affects an estimated 20.7 million Americans, mostly after age 45, with women more commonly affected than men. Physicians make a diagnosis of OA based on a physical exam and history of symptoms. X-rays are used to confirm diagnosis. Most people over 60 reflect the disease on X-ray, and about one-third have actual symptoms.

[0014] There are many factors that can cause OA. Obesity may lead to osteoarthritis of the knees. In addition, people with joint injuries due to sports, work-related activity or accidents may be at increased risk of developing OA.

[0015] Genetics has a role in the development of OA. Some people may be born with defective cartilage or with slight defects in the way that joints fit together. As a person ages, these defects may cause early cartilage breakdown in the joint or the inability to repair damaged or deteriorated cartilage in the joint.

[0016] Inclusion or exclusion of samples for an osteoarthritis pool may be based upon the following criteria: ethnicity (e.g., samples derived from an individual characterized as Caucasian); parental ethnicity (e.g., samples derived from an individual of British paternal and maternal descent); relevant phenotype information for the individual (e.g., case samples derived from individuals diagnosed with specific knee osteoarthritis (OA) and were recruited from an OA knee replacement clinic). Control samples may be selected based on relevant phenotype information for the individual (e.g., derived from individuals free of OA at several sites (knee, hand, hip etc)); and no family history of OA and/or rheumatoid arthritis. Additional phenotype information collected for both cases and controls may include

age of the individual, gender, family history of OA, diagnosis with osteoarthritis (joint location of OA, date of primary diagnosis, age of individual as of primary diagnosis), knee history (current symptoms, any major knee injury, meniscectomy, knee replacement surgery, age of surgery), HRT history, osteoporosis diagnosis.

[0017] Based in part upon selection criteria set forth above, individuals having osteoarthritis can be selected for genetic studies. Also, individuals having no history of osteoarthritis often are selected for genetic studies, as described hereafter.

#### Polymorphic Variants Associated with Osteoarthritis

[0018] A genetic analysis provided herein linked osteoarthritis with polymorphic variant nucleic acid sequences in the human genome. As used herein, the term “polymorphic site” refers to a region in a nucleic acid at which two or more alternative nucleotide sequences are observed in a significant number of nucleic acid samples from a population of individuals. A polymorphic site may be a nucleotide sequence of two or more nucleotides, an inserted nucleotide or nucleotide sequence, a deleted nucleotide or nucleotide sequence, or a microsatellite, for example. A polymorphic site that is two or more nucleotides in length may be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more, 20 or more, 30 or more, 50 or more, 75 or more, 100 or more, 500 or more, or about 1000 nucleotides in length, where all or some of the nucleotide sequences differ within the region. A polymorphic site is often one nucleotide in length, which is referred to herein as a “single nucleotide polymorphism” or a “SNP.”

[0019] Where there are two, three, or four alternative nucleotide sequences at a polymorphic site, each nucleotide sequence is referred to as a “polymorphic variant” or “nucleic acid variant.” Where two polymorphic variants exist, for example, the polymorphic variant represented in a minority of samples from a population is sometimes referred to as a “minor allele” and the polymorphic variant that is more prevalently represented is sometimes referred to as a “major allele.” Many organisms possess a copy of each chromosome (*e.g.*, humans), and those individuals who possess two major alleles or two minor alleles are often referred to as being “homozygous” with respect to the polymorphism, and those individuals who possess one major allele and one minor allele are normally referred to as being “heterozygous” with respect to the polymorphism. Individuals who are homozygous with respect to one allele are sometimes predisposed to a different phenotype as compared to individuals who are heterozygous or homozygous with respect to another allele.

[0020] In genetic analysis that associate polymorphic variants with osteoarthritis, samples from individuals having osteoarthritis and individuals not having osteoarthritis often are allelotyped and/or genotyped. The term “allelotype” as used herein refers to a process for determining the allele frequency for a polymorphic variant in pooled DNA samples from cases and controls. By pooling DNA from each group, an allele frequency for each SNP in each group is calculated. These allele frequencies are then

compared to one another. The term “genotyped” as used herein refers to a process for determining a genotype of one or more individuals, where a “genotype” is a representation of one or more polymorphic variants in a population.

**[0021]** A genotype or polymorphic variant may be expressed in terms of a “haplotype,” which as used herein refers to two or more polymorphic variants occurring within genomic DNA in a group of individuals within a population. For example, two SNPs may exist within a gene where each SNP position includes a cytosine variation and an adenine variation. Certain individuals in a population may carry one allele (heterozygous) or two alleles (homozygous) having the gene with a cytosine at each SNP position. As the two cytosines corresponding to each SNP in the gene travel together on one or both alleles in these individuals, the individuals can be characterized as having a cytosine/cytosine haplotype with respect to the two SNPs in the gene.

**[0022]** As used herein, the term “phenotype” refers to a trait which can be compared between individuals, such as presence or absence of a condition, a visually observable difference in appearance between individuals, metabolic variations, physiological variations, variations in the function of biological molecules, and the like. An example of a phenotype is occurrence of osteoarthritis.

**[0023]** Researchers sometimes report a polymorphic variant in a database without determining whether the variant is represented in a significant fraction of a population. Because a subset of these reported polymorphic variants are not represented in a statistically significant portion of the population, some of them are sequencing errors and/or not biologically relevant. Thus, it is often not known whether a reported polymorphic variant is statistically significant or biologically relevant until the presence of the variant is detected in a population of individuals and the frequency of the variant is determined. Methods for detecting a polymorphic variant in a population are described herein, specifically in Example 2. A polymorphic variant is statistically significant and often biologically relevant if it is represented in 5% or more of a population, sometimes 10% or more, 15% or more, or 20% or more of a population, and often 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, or 50% or more of a population.

**[0024]** A polymorphic variant may be detected on either or both strands of a double-stranded nucleic acid. Also, a polymorphic variant may be located within an intron or exon of a gene or within a portion of a regulatory region such as a promoter, a 5′ untranslated region (UTR), a 3′ UTR, and in DNA (*e.g.*, genomic DNA (gDNA) and complementary DNA (cDNA)), RNA (*e.g.*, mRNA, tRNA, and rRNA), or a polypeptide. Polymorphic variations may or may not result in detectable differences in gene expression, polypeptide structure, or polypeptide function.

**[0025]** It was determined that polymorphic variations associated with an increased risk of osteoarthritis existed in the *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* regions. In certain embodiments, polymorphic variants at positions rs756519, rs1042327, rs8770, rs1563055, rs912428 and rs1888475 in the human genome were associated with an increased risk of osteoarthritis, and in specific

embodiments, a thymine at position rs756519, a cytosine at position rs1042327, a cytosine at position rs8770, a thymine at position rs1563055, a thymine at position rs912428 and an adenine at position rs1888475 were associated with an increased risk of osteoarthritis.

**[0026]** Polymorphic variants in and around the *chrom 6* region were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 1 selected from the group consisting of 229, 6310, 11840, 11870, 12064, 13392, 16354, 16559, 16935, 17616, 17737, 18321, 18453, 18811, 20020, 21662, 23197, 23446, 24339, 25504, 27174, 28008, 29294, 29759, 30832, 44512, 44850, 45884, 46345, 48589, 53371, 53911, 53990, 55152, 55667, 58952, 59315, 60029, 61477, 62988, 63090, 64021, 65685, 70220, 70323, 70959, 73436, 82945, 82958, 82961, 82964, 82965, 83006, 83025, 83034, 83074, 83132, 83155, 83172, 83174, 83206, 83216, 83234, 83252, 83260, 83263, 83296, 83319, 83322, 83324, 83357, 83375, 83381, 83389, 83443, 83499, 83545, 83566, 83591, 83619, 83698, 83780, 83784, 83826, 83832, 83852, 86297, 86315, 86420, 86460, 86714, 86718, 86736, 86753, 86766, 88162, 88218, 88246, 88255, 88309, 88310, 88471, 88619, 88904, 89044, 90531, 90534, 90613 and 46252. Polymorphic variants at the following positions in SEQ ID NO: 1 in particular were associated with an increased risk of osteoarthritis: 229, 6310, 16559, 18453, 25504, 27174, 30832, 44850, 45884, 48589, 61477, 82961 and 46252, with specific embodiments directed to variants at positions 229, 16559, 44850 and/or 46252. In particular, the following polymorphic variants in SEQ ID NO: 1 were associated with risk of osteoarthritis: a thymine at position 229, a guanine at position 6310, a thymine at position 16559, an adenine at position 18453, an adenine at position 25504, an adenine at position 27174, an adenine at position 30832, a guanine at position 44850, an adenine at position 45884, an adenine at position 48589, a cytosine at position 61477, a cytosine at position 82961 and a thymine at position 46252.

**[0027]** Polymorphic variants in and around the *ELP3* region were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 2 selected from the group consisting of 211, 473, 1536, 5639, 17186, 17335, 25029, 25111, 28811, 28863, 30809, 40985, 45147, 45282, 46168, 46328, 49077, 51925, 52141, 52168, 60852, 62468, 65572, 79089, 79541, 79790, 90843, 90978, 91052, 91131, 91132, 94439 and 94621. Polymorphic variants at the following positions in SEQ ID NO: 2 in particular were associated with an increased risk of osteoarthritis: 40985, 46168, 51925 and 52168. In particular, the following polymorphic variants in SEQ ID NO: 2 were associated with risk of osteoarthritis: a cytosine at position 40985, a guanine at position 46168, a thymine at position 51925 and a cytosine at position 52168.

**[0028]** Polymorphic variants in and around the *CHDC1* region were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 3 selected from the group consisting of 243, 10208, 15049, 15111, 15272, 15287, 15326, 15327, 17038, 19391, 21702, 22431, 22881, 27744, 32564, 32698, 33104, 33181, 33256, 33543, 35567, 40085, 40482, 45641, 46059, 48504, 48919, 49693, 49874, 50020, 50616, 50719, 55511, 65533, 70529, 75591, 77266, 80368, 82475, 92462,

92480, 95819 and 96275. Polymorphic variants at the following positions in SEQ ID NO: 3 in particular were associated with an increased risk of osteoarthritis: 15111, 45641, 46059, 49693, 49874, 50020, 50719, 70529, 82475, 92462, 92480 and 96275, with specific embodiments directed to variants at positions 82475 and/or 92462. In particular, the following polymorphic variants in SEQ ID NO: 3 were associated with risk of osteoarthritis: a guanine at position 15111, a thymine at position 45641, an adenine at position 46059, a cytosine at position 49693, an adenine at position 49874, an adenine at position 50020, a guanine at position 50719, an adenine at position 70529, an adenine at position 82475, a thymine at position 92462, a thymine at position 92480 and a cytosine at position 96275.

**[0029]** Polymorphic variants in and around the *ERG* region were tested for association with osteoarthritis. These include polymorphic variants at positions in SEQ ID NO: 4 selected from the group consisting of 231, 882, 960, 1194, 1530, 1673, 2096, 2285, 5873, 7256, 7988, 8222, 8381, 8814, 8915, 9642, 9902, 10619, 10927, 11032, 14377, 15608, 15928, 16296, 17598, 19272, 20084, 20577, 28051, 29466, 29530, 29987, 30012, 30322, 32216, 32516, 32544, 32746, 33137, 33538, 33798, 33802, 33964, 34132, 34210, 34317, 34499, 34753, 34845, 35335, 36423, 36450, 36481, 38447, 38784, 39387, 39458, 39822, 40305, 40869, 40926, 41010, 41134, 41984, 42172, 42753, 43011, 43176, 43320, 43381, 44142, 44383, 44726, 45087, 45141, 45359, 45421, 45456, 45467, 45486, 45709, 45716, 47626, 49413, 49796, 49962, 50075, 50093, 50571, 50615, 50780, 50851, 51459, 53193, 53702, 53736, 53795, 54109, 54126, 54230, 54894, 55455, 55499, 56522, 56662, 56954, 57267, 58282, 58916, 59544, 59666, 59913, 66846, 67245, 67652, 67955, 67966, 68420, 70226, 70810, 72246, 73330, 73457, 74389, 74638, 74640, 75358, 75952, 76098, 77836, 78449, 78507, 80031, 81695, 82775, 82795, 84611, 84657, 84693, 85020, 85048, 85100, 85325, 85452, 85868, 85936, 85990, 86139, 86497, 87236, 87248, 87533, 87912, 88108, 88494, 89598, 90235, 91287, 91359, 92384, 92410, 92900, 94495, 94512, 97777 and 98333. Polymorphic variants at the following positions in SEQ ID NO: 4 in particular were associated with an increased risk of osteoarthritis: 1673, 20577, 33137, 39822, 45716, 49962, 51459, 54894, 55455, 55499, 58282, 68420 and 80031, with specific embodiments directed to variants at positions 33137, 55499 and/or 58282. In particular, the following polymorphic variants in SEQ ID NO: 4 were associated with risk of osteoarthritis: a guanine at position 1673, a thymine at position 20577, a guanine at position 33137, a guanine at position 39822, an adenine at position 45716, a guanine at position 49962, an adenine at position 51459, a cytosine at position 54894, an adenine at position 55455, an adenine at position 55499, a guanine at position 58282, an adenine at position 68420 and a thymine at position 80031.

**[0030]** Based in part upon analyses summarized in Figures 1A-1D, regions with significant association have been identified in loci associated with osteoarthritis. Any polymorphic variants associated with osteoarthritis in a region of significant association can be utilized for embodiments described herein. For example, polymorphic variants in a region spanning chromosome positions 170719500 to 170766500 (approximately 47,000 nucleotides in length) in a *chrom 6* region, spanning

chromosome positions 27963000 to 27983000 (approximately 20,000 nucleotides in length) in a *ELP3* region, spanning chromosome positions 44962000 to 45013000 (approximately 51,000 nucleotides in length) in a *CHDC1* region, and spanning chromosome positions 38830000 to 38844000 (approximately 14,000 nucleotides in length) in a *ERG* region, have significant association (chromosome positions are within NCBI's Genome build 34).

#### Additional Polymorphic Variants Associated with Osteoarthritis

[0031] Also provided is a method for identifying polymorphic variants proximal to an incident, founder polymorphic variant associated with osteoarthritis. Thus, featured herein are methods for identifying a polymorphic variation associated with osteoarthritis that is proximal to an incident polymorphic variation associated with osteoarthritis, which comprises identifying a polymorphic variant proximal to the incident polymorphic variant associated with osteoarthritis, where the incident polymorphic variant is in a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence. The nucleotide sequence often comprises a polynucleotide sequence selected from the group consisting of (a) a polynucleotide sequence of SEQ ID NO: 1-12; (b) a polynucleotide sequence that encodes a polypeptide having an amino acid sequence encoded by a polynucleotide sequence of SEQ ID NO: 1-12; and (c) a polynucleotide sequence that encodes a polypeptide having an amino acid sequence that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1 or a polynucleotide sequence 90% or more identical to the polynucleotide sequence of SEQ ID NO: 1-12. The presence or absence of an association of the proximal polymorphic variant with osteoarthritis then is determined using a known association method, such as a method described in the Examples hereafter. In an embodiment, the incident polymorphic variant is a polymorphic variant associated with osteoarthritis described herein. In another embodiment, the proximal polymorphic variant identified sometimes is a publicly disclosed polymorphic variant, which for example, sometimes is published in a publicly available database. In other embodiments, the polymorphic variant identified is not publicly disclosed and is discovered using a known method, including, but not limited to, sequencing a region surrounding the incident polymorphic variant in a group of nucleic samples. Thus, multiple polymorphic variants proximal to an incident polymorphic variant are associated with osteoarthritis using this method.

[0032] The proximal polymorphic variant often is identified in a region surrounding the incident polymorphic variant. In certain embodiments, this surrounding region is about 50 kb flanking the first polymorphic variant (e.g. about 50 kb 5' of the first polymorphic variant and about 50 kb 3' of the first polymorphic variant), and the region sometimes is composed of shorter flanking sequences, such as flanking sequences of about 40 kb, about 30 kb, about 25 kb, about 20 kb, about 15 kb, about 10 kb, about 7 kb, about 5 kb, or about 2 kb 5' and 3' of the incident polymorphic variant. In other embodiments, the region is composed of longer flanking sequences, such as flanking sequences of about

55 kb, about 60 kb, about 65 kb, about 70 kb, about 75 kb, about 80 kb, about 85 kb, about 90 kb, about 95 kb, or about 100 kb 5' and 3' of the incident polymorphic variant.

[0033] In certain embodiments, polymorphic variants associated with osteoarthritis are identified iteratively. For example, a first proximal polymorphic variant is associated with osteoarthritis using the methods described above and then another polymorphic variant proximal to the first proximal polymorphic variant is identified (*e.g.*, publicly disclosed or discovered) and the presence or absence of an association of one or more other polymorphic variants proximal to the first proximal polymorphic variant with osteoarthritis is determined.

[0034] The methods described herein are useful for identifying or discovering additional polymorphic variants that may be used to further characterize a gene, region or loci associated with a condition, a disease (*e.g.*, osteoarthritis), or a disorder. For example, allelotyping or genotyping data from the additional polymorphic variants may be used to identify a functional mutation or a region of linkage disequilibrium. In certain embodiments, polymorphic variants identified or discovered within a region comprising the first polymorphic variant associated with osteoarthritis are genotyped using the genetic methods and sample selection techniques described herein, and it can be determined whether those polymorphic variants are in linkage disequilibrium with the first polymorphic variant. The size of the region in linkage disequilibrium with the first polymorphic variant also can be assessed using these genotyping methods. Thus, provided herein are methods for determining whether a polymorphic variant is in linkage disequilibrium with a first polymorphic variant associated with osteoarthritis, and such information can be used in prognosis/diagnosis methods described herein.

#### Isolated Nucleic Acids

[0035] Featured herein are isolated *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid variants depicted in SEQ ID NO: 1-12, and substantially identical nucleic acids thereof. A nucleic acid variant may be represented on one or both strands in a double-stranded nucleic acid or on one chromosomal complement (heterozygous) or both chromosomal complements (homozygous).

[0036] As used herein, the term "nucleic acid" includes DNA molecules (*e.g.*, a complementary DNA (cDNA) and genomic DNA (gDNA)) and RNA molecules (*e.g.*, mRNA, rRNA, siRNA and tRNA) and analogs of DNA or RNA, for example, by use of nucleotide analogs. The nucleic acid molecule can be single-stranded and it is often double-stranded. The term "isolated or purified nucleic acid" refers to nucleic acids that are separated from other nucleic acids present in the natural source of the nucleic acid. For example, with regard to genomic DNA, the term "isolated" includes nucleic acids which are separated from the chromosome with which the genomic DNA is naturally associated. An "isolated" nucleic acid is often free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic

acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. As used herein, the term "gene" refers to a nucleotide sequence that encodes a polypeptide.

[0037] Also included herein are nucleic acid fragments. These fragments often have a nucleotide sequence identical to a nucleotide sequence of SEQ ID NO: 1-12, a nucleotide sequence substantially identical to a nucleotide sequence of SEQ ID NO: 1-12, or a nucleotide sequence that is complementary to the foregoing. The nucleic acid fragment may be identical, substantially identical or homologous to a nucleotide sequence in an exon or an intron in a nucleotide sequence of SEQ ID NO: 1-12, and may encode a domain or part of a domain of a polypeptide. Sometimes, the fragment will comprises one or more of the polymorphic variations described herein as being associated with osteoarthritis. The nucleic acid fragment is often 50, 100, or 200 or fewer base pairs in length, and is sometimes about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 2000, 3000, 4000, 5000, 10000, 15000, or 20000 base pairs in length. A nucleic acid fragment that is complementary to a nucleotide sequence identical or substantially identical to a nucleotide sequence in SEQ ID NO: 1-12 and hybridizes to such a nucleotide sequence under stringent conditions is often referred to as a "probe." Nucleic acid fragments often include one or more polymorphic sites, or sometimes have an end that is adjacent to a polymorphic site as described hereafter.

[0038] An example of a nucleic acid fragment is an oligonucleotide. As used herein, the term "oligonucleotide" refers to a nucleic acid comprising about 8 to about 50 covalently linked nucleotides, often comprising from about 8 to about 35 nucleotides, and more often from about 10 to about 25 nucleotides. The backbone and nucleotides within an oligonucleotide may be the same as those of naturally occurring nucleic acids, or analogs or derivatives of naturally occurring nucleic acids, provided that oligonucleotides having such analogs or derivatives retain the ability to hybridize specifically to a nucleic acid comprising a targeted polymorphism. Oligonucleotides described herein may be used as hybridization probes or as components of prognostic or diagnostic assays, for example, as described herein.

[0039] Oligonucleotides are typically synthesized using standard methods and equipment, such as the ABI™3900 High Throughput DNA Synthesizer and the EXPEDITE™ 8909 Nucleic Acid Synthesizer, both of which are available from Applied Biosystems (Foster City, CA). Analogs and derivatives are exemplified in U.S. Pat. Nos. 4,469,863; 5,536,821; 5,541,306; 5,637,683; 5,637,684;

5,700,922; 5,717,083; 5,719,262; 5,739,308; 5,773,601; 5,886,165; 5,929,226; 5,977,296; 6,140,482; WO 00/56746; WO 01/14398, and related publications. Methods for synthesizing oligonucleotides comprising such analogs or derivatives are disclosed, for example, in the patent publications cited above and in U.S. Pat. Nos. 5,614,622; 5,739,314; 5,955,599; 5,962,674; 6,117,992; in WO 00/75372; and in related publications.

**[0040]** Oligonucleotides may also be linked to a second moiety. The second moiety may be an additional nucleotide sequence such as a tail sequence (*e.g.*, a polyadenosine tail), an adapter sequence (*e.g.*, phage M13 universal tail sequence), and others. Alternatively, the second moiety may be a non-nucleotide moiety such as a moiety which facilitates linkage to a solid support or a label to facilitate detection of the oligonucleotide. Such labels include, without limitation, a radioactive label, a fluorescent label, a chemiluminescent label, a paramagnetic label, and the like. The second moiety may be attached to any position of the oligonucleotide, provided the oligonucleotide can hybridize to the nucleic acid comprising the polymorphism.

#### Uses for Nucleic Acid Sequence

**[0041]** Nucleic acid coding sequences may be used for diagnostic purposes for detection and control of polypeptide expression. Also, included herein are oligonucleotide sequences such as antisense RNA, small-interfering RNA (siRNA) and DNA molecules and ribozymes that function to inhibit translation of a polypeptide. Antisense techniques and RNA interference techniques are known in the art and are described herein.

**[0042]** Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, hammerhead motif ribozyme molecules may be engineered that specifically and efficiently catalyze endonucleolytic cleavage of RNA sequences corresponding to or complementary to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between fifteen (15) and twenty (20) ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

**[0043]** Antisense RNA and DNA molecules, siRNA and ribozymes may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically

synthesizing oligodeoxyribonucleotides well known in the art such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

[0044] DNA encoding a polypeptide also may have a number of uses for the diagnosis of diseases, including osteoarthritis, resulting from aberrant expression of a target gene described herein. For example, the nucleic acid sequence may be used in hybridization assays of biopsies or autopsies to diagnose abnormalities of expression or function (*e.g.*, Southern or Northern blot analysis, *in situ* hybridization assays).

[0045] In addition, the expression of a polypeptide during embryonic development may also be determined using nucleic acid encoding the polypeptide. As addressed, *infra*, production of functionally impaired polypeptide is the cause of various disease states, such as osteoarthritis. *In situ* hybridizations using polypeptide as a probe may be employed to predict problems related to osteoarthritis. Further, as indicated, *infra*, administration of human active polypeptide, recombinantly produced as described herein, may be used to treat disease states related to functionally impaired polypeptide. Alternatively, gene therapy approaches may be employed to remedy deficiencies of functional polypeptide or to replace or compete with dysfunctional polypeptide.

#### Expression Vectors, Host Cells, and Genetically Engineered Cells

[0046] Provided herein are nucleic acid vectors, often expression vectors, which contain a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence, or a substantially identical sequence thereof. As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid, or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors may include replication defective retroviruses, adenoviruses and adeno-associated viruses for example.

[0047] A vector can include a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence in a form suitable for expression of an encoded target polypeptide or target nucleic acid in a host cell. A “target polypeptide” is a polypeptide encoded by a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence, or a substantially identical nucleotide sequence thereof. The recombinant expression vector typically includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term “regulatory sequence” includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or

inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, and the like. Expression vectors can be introduced into host cells to produce target polypeptides, including fusion polypeptides.

[0048] Recombinant expression vectors can be designed for expression of target polypeptides in prokaryotic or eukaryotic cells. For example, target polypeptides can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology 185*, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[0049] Expression of polypeptides in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant polypeptide; 2) to increase the solubility of the recombinant polypeptide; and 3) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion polypeptide. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith & Johnson, *Gene 67*: 31-40 (1988)), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding polypeptide, or polypeptide A, respectively, to the target recombinant polypeptide.

[0050] Purified fusion polypeptides can be used in screening assays and to generate antibodies specific for target polypeptides. In a therapeutic embodiment, fusion polypeptide expressed in a retroviral expression vector is used to infect bone marrow cells that are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

[0051] Expressing the polypeptide in host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide is often used to maximize recombinant polypeptide expression (Gottesman, S., *Gene Expression Technology: Methods in Enzymology, Academic Press, San Diego, California 185*: 119-128 (1990)). Another strategy is to alter the nucleotide sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, *Nucleic Acids Res. 20*: 2111-2118 (1992)). Such alteration of nucleotide sequences can be carried out by standard DNA synthesis techniques.

**[0052]** When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. Recombinant mammalian expression vectors are often capable of directing expression of the nucleic acid in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include an albumin promoter (liver-specific; Pinkert *et al.*, *Genes Dev.* 1: 268-277 (1987)), lymphoid-specific promoters (Calame & Eaton, *Adv. Immunol.* 43: 235-275 (1988)), promoters of T cell receptors (Winoto & Baltimore, *EMBO J.* 8: 729-733 (1989)) promoters of immunoglobulins (Banerji *et al.*, *Cell* 33: 729-740 (1983); Queen & Baltimore, *Cell* 33: 741-748 (1983)), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne & Ruddle, *Proc. Natl. Acad. Sci. USA* 86: 5473-5477 (1989)), pancreas-specific promoters (Edlund *et al.*, *Science* 230: 912-916 (1985)), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are sometimes utilized, for example, the murine hox promoters (Kessel & Gruss, *Science* 249: 374-379 (1990)) and the  $\alpha$ -fetoprotein promoter (Campes & Tilghman, *Genes Dev.* 3: 537-546 (1989)).

**[0053]** A *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid also may be cloned into an expression vector in an antisense orientation. Regulatory sequences (*e.g.*, viral promoters and/or enhancers) operatively linked to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid cloned in the antisense orientation can be chosen for directing constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. Antisense expression vectors can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub *et al.*, *Antisense RNA as a molecular tool for genetic analysis, Reviews - Trends in Genetics*, Vol. 1(1) (1986).

**[0054]** Also provided herein are host cells that include a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence within a recombinant expression vector or a fragment of such a nucleotide sequence which facilitate homologous recombination into a specific site of the host cell genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but rather also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell can be any prokaryotic or eukaryotic cell. For example, a target polypeptide can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

[0055] Vectors can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms “transformation” and “transfection” are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, transduction/infection, DEAE-dextran-mediated transfection, lipofection, or electroporation.

[0056] A host cell provided herein can be used to produce (*i.e.*, express) a target polypeptide or a substantially identical polypeptide thereof. Accordingly, further provided are methods for producing a target polypeptide using host cells described herein. In one embodiment, the method includes culturing host cells into which a recombinant expression vector encoding a target polypeptide has been introduced in a suitable medium such that a target polypeptide is produced. In another embodiment, the method further includes isolating a target polypeptide from the medium or the host cell.

[0057] Also provided are cells or purified preparations of cells which include a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* transgene, or which otherwise misexpress target polypeptide. Cell preparations can consist of human or non-human cells, *e.g.*, rodent cells, *e.g.*, mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* transgene (*e.g.*, a heterologous form of a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* gene, such as a human gene expressed in non-human cells). The transgene can be misexpressed, *e.g.*, overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous target polypeptide (*e.g.*, expression of a gene is disrupted, also known as a knockout). Such cells can serve as a model for studying disorders which are related to mutated or mis-expressed alleles or for use in drug screening. Also provided are human cells (*e.g.*, a hematopoietic stem cells) transfected with a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid.

[0058] Also provided are cells or a purified preparation thereof (*e.g.*, human cells) in which an endogenous *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid is under the control of a regulatory sequence that does not normally control the expression of the endogenous gene. The expression characteristics of an endogenous gene within a cell (*e.g.*, a cell line or microorganism) can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the corresponding endogenous gene. For example, an endogenous corresponding gene (*e.g.*, a gene which is “transcriptionally silent,” not normally expressed, or expressed only at very low levels) may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, *e.g.*, Chappel, US 5,272,071; WO 91/06667, published on May 16, 1991.

### Transgenic Animals

[0059] Non-human transgenic animals that express a heterologous target polypeptide (*e.g.*, expressed from a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid or substantially identical sequence thereof) can be generated. Such animals are useful for studying the function and/or activity of a target polypeptide and for identifying and/or evaluating modulators of the activity of a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid or encoded polypeptide. As used herein, a “transgenic animal” is a non-human animal such as a mammal (*e.g.*, a non-human primate such as chimpanzee, baboon, or macaque; an ungulate such as an equine, bovine, or caprine; or a rodent such as a rat, a mouse, or an Israeli sand rat), a bird (*e.g.*, a chicken or a turkey), an amphibian (*e.g.*, a frog, salamander, or newt), or an insect (*e.g.*, *Drosophila melanogaster*), in which one or more of the cells of the animal includes a transgene. A transgene is exogenous DNA or a rearrangement (*e.g.*, a deletion of endogenous chromosomal DNA) that is often integrated into or occurs in the genome of cells in a transgenic animal. A transgene can direct expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, and other transgenes can reduce expression (*e.g.*, a knockout). Thus, a transgenic animal can be one in which an endogenous nucleic acid homologous to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal (*e.g.*, an embryonic cell of the animal) prior to development of the animal.

[0060] Intronic sequences and polyadenylation signals can also be included in the transgene to increase expression efficiency of the transgene. One or more tissue-specific regulatory sequences can be operably linked to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence to direct expression of an encoded polypeptide to particular cells. A transgenic founder animal can be identified based upon the presence of a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence in its genome and/or expression of encoded mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence can further be bred to other transgenic animals carrying other transgenes.

[0061] Target polypeptides can be expressed in transgenic animals or plants by introducing, for example, a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid into the genome of an animal that encodes the target polypeptide. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, *e.g.*, a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Also included is a population of cells from a transgenic animal.

### Target Polypeptides

**[0062]** Also featured herein are isolated target polypeptides, which are encoded by a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence (e.g., SEQ ID NO: 1-12), or a substantially identical nucleotide sequence thereof. Examples of *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* polypeptides are set forth in SEQ ID NO: 13-20. The term “polypeptide” as used herein includes proteins and peptides. An “isolated” or “purified” polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language “substantially free” means preparation of a target polypeptide having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-target polypeptide (also referred to herein as a “contaminating protein”), or of chemical precursors or non-target chemicals. When the target polypeptide or a biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, specifically, where culture medium represents less than about 20%, sometimes less than about 10%, and often less than about 5% of the volume of the polypeptide preparation. Isolated or purified target polypeptide preparations are sometimes 0.01 milligrams or more or 0.1 milligrams or more, and often 1.0 milligrams or more and 10 milligrams or more in dry weight.

**[0063]** Further included herein are target polypeptide fragments. The polypeptide fragment may be a domain or part of a domain of a target polypeptide. The polypeptide fragment may have increased, decreased or unexpected biological activity. The polypeptide fragment is often 50 or fewer, 100 or fewer, or 200 or fewer amino acids in length, and is sometimes 300, 400, 500, 600, 700, or 900 or fewer amino acids in length. Certain embodiments are directed to *PSMB1* polypeptide fragments (e.g., sequence accessed by NP\_002784; rs756519 in Table A), such as a proteasome protease domain (e.g., starting at about amino acid 34 and ending at about amino acid 226) or a proteasome B domain (e.g., starting at about amino acid 41 and ending at about amino acid 88).

**[0064]** Substantially identical target polypeptides may depart from the amino acid sequences of target polypeptides in different manners. For example, conservative amino acid modifications may be introduced at one or more positions in the amino acid sequences of target polypeptides. A “conservative amino acid substitution” is one in which the amino acid is replaced by another amino acid having a similar structure and/or chemical function. Families of amino acid residues having similar structures and functions are well known. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine,

tryptophan, histidine). Also, essential and non-essential amino acids may be replaced. A “non-essential” amino acid is one that can be altered without abolishing or substantially altering the biological function of a target polypeptide, whereas altering an “essential” amino acid abolishes or substantially alters the biological function of a target polypeptide. Amino acids that are conserved among target polypeptides are typically essential amino acids. In certain embodiments, the polypeptide includes one or more non-synonymous polymorphic variants associated with osteoarthritis.

[0065] Also, target polypeptides may exist as chimeric or fusion polypeptides. As used herein, a target “chimeric polypeptide” or target “fusion polypeptide” includes a target polypeptide linked to a non-target polypeptide. A “non-target polypeptide” refers to a polypeptide having an amino acid sequence corresponding to a polypeptide which is not substantially identical to the target polypeptide, which includes, for example, a polypeptide that is different from the target polypeptide and derived from the same or a different organism. The target polypeptide in the fusion polypeptide can correspond to an entire or nearly entire target polypeptide or a fragment thereof. The non-target polypeptide can be fused to the N-terminus or C-terminus of the target polypeptide.

[0066] Fusion polypeptides can include a moiety having high affinity for a ligand. For example, the fusion polypeptide can be a GST-target fusion polypeptide in which the target sequences are fused to the C-terminus of the GST sequences, or a polyhistidine-target fusion polypeptide in which the target polypeptide is fused at the N- or C-terminus to a string of histidine residues. Such fusion polypeptides can facilitate purification of recombinant target polypeptide. Expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide), and a nucleotide sequence in SEQ ID NO: 1-12, or a substantially identical nucleotide sequence thereof, can be cloned into an expression vector such that the fusion moiety is linked in-frame to the target polypeptide. Further, the fusion polypeptide can be a target polypeptide containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression, secretion, cellular internalization, and cellular localization of a target polypeptide can be increased through use of a heterologous signal sequence. Fusion polypeptides can also include all or a part of a serum polypeptide (*e.g.*, an IgG constant region or human serum albumin).

[0067] Target polypeptides can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. Administration of these target polypeptides can be used to affect the bioavailability of a substrate of the target polypeptide and may effectively increase target polypeptide biological activity in a cell. Target fusion polypeptides may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a target polypeptide; (ii) misregulation of the gene encoding the target polypeptide; and (iii) aberrant post-translational modification of a target polypeptide. Also, target polypeptides can be used as immunogens to produce anti-target

antibodies in a subject, to purify target polypeptide ligands or binding partners, and in screening assays to identify molecules which inhibit or enhance the interaction of a target polypeptide with a substrate.

**[0068]** In addition, polypeptides can be chemically synthesized using techniques known in the art (See, *e.g.*, Creighton, 1983 *Proteins*. New York, N.Y.: W. H. Freeman and Company; and Hunkapiller et al., (1984) *Nature* July 12 -18;310(5973):105-11). For example, a relative short fragment can be synthesized by use of a peptide synthesizer. Furthermore, if desired, non-classical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the fragment sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu,  $\epsilon$ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoroamino acids, designer amino acids such as  $\beta$ -methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

**[0069]** Polypeptides and polypeptide fragments sometimes are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH<sub>4</sub>; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; and the like. Additional post-translational modifications include, for example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptide fragments may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the polypeptide.

**[0070]** Also provided are chemically modified derivatives of polypeptides that can provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (*see e.g.*, U.S. Pat. No: 4,179,337. The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

**[0071]** The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term

“about” indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

[0072] The polymers should be attached to the polypeptide with consideration of effects on functional or antigenic domains of the polypeptide. There are a number of attachment methods available to those skilled in the art (e.g., EP 0 401 384 (coupling PEG to G-CSF) and Malik et al. (1992) *Exp Hematol.* September;20(8):1028-35 (pegylation of GM-CSF using tresyl chloride)). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. For therapeutic purposes, the attachment sometimes is at an amino group, such as attachment at the N-terminus or lysine group.

[0073] Proteins can be chemically modified at the N-terminus. Using polyethylene glycol as an illustration of such a composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, and the like), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus may be accomplished by reductive alkylation, which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

#### Substantially Identical Nucleic Acids and Polypeptides

[0074] Nucleotide sequences and polypeptide sequences that are substantially identical to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence and the target polypeptide sequences encoded by those nucleotide sequences, respectively, are included herein. The term “substantially identical” as used herein refers to two or more nucleic acids or polypeptides sharing one or more identical nucleotide sequences or polypeptide sequences, respectively. Included are nucleotide sequences or polypeptide

sequences that are 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more (each often within a 1%, 2%, 3% or 4% variability) identical to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence or the encoded target polypeptide amino acid sequences. One test for determining whether two nucleic acids are substantially identical is to determine the percent of identical nucleotide sequences or polypeptide sequences shared between the nucleic acids or polypeptides.

[0075] Calculations of sequence identity are often performed as follows. Sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The length of a reference sequence aligned for comparison purposes is sometimes 30% or more, 40% or more, 50% or more, often 60% or more, and more often 70% or more, 80% or more, 90% or more, or 100% of the length of the reference sequence. The nucleotides or amino acids at corresponding nucleotide or polypeptide positions, respectively, are then compared among the two sequences. When a position in the first sequence is occupied by the same nucleotide or amino acid as the corresponding position in the second sequence, the nucleotides or amino acids are deemed to be identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, introduced for optimal alignment of the two sequences.

[0076] Comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. Percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of Meyers & Miller, *CABIOS* 4: 11-17 (1989), which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. Also, percent identity between two amino acid sequences can be determined using the Needleman & Wunsch, *J. Mol. Biol.* 48: 444-453 (1970) algorithm which has been incorporated into the GAP program in the GCG software package (available at the http address [www.gcg.com](http://www.gcg.com)), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. Percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http address [www.gcg.com](http://www.gcg.com)), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A set of parameters often used is a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0077] Another manner for determining if two nucleic acids are substantially identical is to assess whether a polynucleotide homologous to one nucleic acid will hybridize to the other nucleic acid under stringent conditions. As use herein, the term "stringent conditions" refers to conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in *Current*

*Protocols in Molecular Biology*, John Wiley & Sons, N.Y. , 6.3.1-6.3.6 (1989). Aqueous and non-aqueous methods are described in that reference and either can be used. An example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Often, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. More often, stringency conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C.

**[0078]** An example of a substantially identical nucleotide sequence to a nucleotide sequence in SEQ ID NO: 1-12 is one that has a different nucleotide sequence but still encodes the same polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO: 1-12. Another example is a nucleotide sequence that encodes a polypeptide having a polypeptide sequence that is more than 70% or more identical to, sometimes more than 75% or more, 80% or more, or 85% or more identical to, and often more than 90% or more and 95% or more identical to a polypeptide sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12.

**[0079]** Nucleotide sequences in SEQ ID NO: 1-12 and amino acid sequences of encoded polypeptides can be used as “query sequences” to perform a search against public databases to identify other family members or related sequences, for example. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.*, *J. Mol. Biol.* 215: 403-10 (1990). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleotide sequences in SEQ ID NO: 1-12. BLAST polypeptide searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to polypeptides encoded by the nucleotide sequences of SEQ ID NO: 1-12. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, *Nucleic Acids Res.* 25(17): 3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (*see* the http address [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

**[0080]** A nucleic acid that is substantially identical to a nucleotide sequence in SEQ ID NO: 1 may include polymorphic sites at positions equivalent to those described herein when the sequences are aligned. For example, using the alignment procedures described herein, SNPs in a sequence substantially identical to a sequence in SEQ ID NO: 1-12 can be identified at nucleotide positions that match (*i.e.*,

align) with nucleotides at SNP positions in each nucleotide sequence in SEQ ID NO: 1-12. Also, where a polymorphic variation results in an insertion or deletion, insertion or deletion of a nucleotide sequence from a reference sequence can change the relative positions of other polymorphic sites in the nucleotide sequence.

**[0081]** Substantially identical nucleotide and polypeptide sequences include those that are naturally occurring, such as allelic variants (same locus), splice variants, homologs (different locus), and orthologs (different organism) or can be non-naturally occurring. Non-naturally occurring variants can be generated by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product). Orthologs, homologs, allelic variants, and splice variants can be identified using methods known in the art. These variants normally comprise a nucleotide sequence encoding a polypeptide that is 50% or more, about 55% or more, often about 70-75% or more or about 80-85% or more, and sometimes about 90-95% or more identical to the amino acid sequences of target polypeptides or a fragment thereof. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions to a nucleotide sequence in SEQ ID NO: 1-12 or a fragment of this sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of a nucleotide sequence in SEQ ID NO: 1-12 can further be identified by mapping the sequence to the same chromosome or locus as the nucleotide sequence in SEQ ID NO: 1-12.

**[0082]** Also, substantially identical nucleotide sequences may include codons that are altered with respect to the naturally occurring sequence for enhancing expression of a target polypeptide in a particular expression system. For example, the nucleic acid can be one in which one or more codons are altered, and often 10% or more or 20% or more of the codons are altered for optimized expression in bacteria (*e.g.*, *E. coli*), yeast (*e.g.*, *S. cerevisiae*), human (*e.g.*, 293 cells), insect, or rodent (*e.g.*, hamster) cells.

#### Methods for Identifying Risk of osteoarthritis

**[0083]** Methods for prognosing and diagnosing osteoarthritis are included herein. These methods include detecting the presence or absence of one or more polymorphic variations in a nucleotide sequence associated with osteoarthritis, such as variants in or around the loci set forth herein, or a substantially identical sequence thereof, in a sample from a subject, where the presence of a polymorphic variant described herein is indicative of a risk of osteoarthritis. Determining a risk of osteoarthritis sometimes refers to determining whether an individual is at an increased risk of osteoarthritis (*e.g.*, intermediate risk or higher risk).

**[0084]** Thus, featured herein is a method for identifying a subject who is at risk of osteoarthritis, which comprises detecting an aberration associated with osteoarthritis in a nucleic acid sample from the subject. An embodiment is a method for detecting a risk of osteoarthritis in a subject, which comprises detecting the presence or absence of a polymorphic variation associated with osteoarthritis at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject, where the nucleotide sequence comprises a polynucleotide sequence selected from the group consisting of: (a) a nucleotide sequence of SEQ ID NO: 1-12; (b) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-12; (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-12, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO: 1-12; and (d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising the polymorphic site; whereby the presence of the polymorphic variation is indicative of a predisposition to osteoarthritis in the subject. In certain embodiments, polymorphic variants at the positions described herein are detected for determining a risk of osteoarthritis, and polymorphic variants at positions in linkage disequilibrium with these positions are detected for determining a risk of osteoarthritis. As used herein, "SEQ ID NO: 1-12" refers to individual sequences of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12, each sequence being separately applicable to embodiments described herein.

**[0085]** Risk of osteoarthritis sometimes is expressed as a probability, such as an odds ratio, percentage, or risk factor. Risk often is based upon the presence or absence of one or more polymorphic variants described herein, and also may be based in part upon phenotypic traits of the individual being tested. Methods for calculating risk based upon patient data are well known (*see, e.g., Agresti, Categorical Data Analysis*, 2nd Ed. 2002. Wiley). Allelotyping and genotyping analyses may be carried out in populations other than those exemplified herein to enhance the predictive power of the prognostic method. These further analyses are executed in view of the exemplified procedures described herein, and may be based upon the same polymorphic variations or additional polymorphic variations.

**[0086]** In certain embodiments, determining the presence of a combination of two or more polymorphic variants associated with osteoarthritis in one or more genetic loci (e.g., one or more genes) of the sample is determined to identify, quantify and/or estimate, risk of osteoarthritis. The risk often is the probability of having or developing osteoarthritis. The risk sometimes is expressed as a relative risk with respect to a population average risk of osteoarthritis, and sometimes is expressed as a relative risk with respect to the lowest risk group. Such relative risk assessments often are based upon penetrance values determined by statistical methods, and are particularly useful to clinicians and insurance companies for assessing risk of osteoarthritis (e.g., a clinician can target appropriate detection, prevention and therapeutic regimens to a patient after determining the patient's risk of osteoarthritis, and an insurance company can fine tune actuarial tables based upon population genotype assessments of

osteoarthritis risk). Risk of osteoarthritis sometimes is expressed as an odds ratio, which is the odds of a particular person having a genotype has or will develop osteoarthritis with respect to another genotype group (e.g., the most disease protective genotype or population average). In related embodiments, the determination is utilized to identify a subject at risk of osteoarthritis. In an embodiment, two or more polymorphic variations are detected in two or more regions in human genomic DNA associated with increased risk of osteoarthritis, such as a locus containing a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG*, for example. In certain embodiments, 3 or more, or 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more polymorphic variants are detected in the sample. In specific embodiments, polymorphic variants are detected in a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* region, for example. In another embodiment, polymorphic variants are detected at two or more positions selected from the group consisting of rs756519, rs1042327, rs8770, rs1563055, rs912428 and rs1888475; 229, 16559, 44850 and/or 46252 in SEQ ID NO: 1; 40985, 46168, 51925 and/or 52168 in SEQ ID NO: 2; 49693, 82475, 92462 and/or 96275 in SEQ ID NO: 3; and 33137, 55499 and/or 58282 in SEQ ID NO: 4. In certain embodiments, polymorphic variants are detected at other genetic loci (e.g., the polymorphic variants can be detected in *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* in addition to other loci or only in other loci), where the other loci include but are not limited to those described in concurrently-filed patent applications having attorney docket number 524593008800, 524593008900, 524593009000, 524593009100 or 524593009200, each of which is incorporated herein by reference in its entirety.

**[0087]** Results from prognostic tests may be combined with other test results to diagnose osteoarthritis. For example, prognostic results may be gathered, a patient sample may be ordered based on a determined predisposition to osteoarthritis, the patient sample is analyzed, and the results of the analysis may be utilized to diagnose osteoarthritis. Also osteoarthritis diagnostic method can be developed from studies used to generate prognostic methods in which populations are stratified into subpopulations having different progressions of osteoarthritis. In another embodiment, prognostic results may be gathered, a patient's risk factors for developing osteoarthritis (e.g., age, weight, race, diet) analyzed, and a patient sample may be ordered based on a determined predisposition to osteoarthritis.

**[0088]** The nucleic acid sample typically is isolated from a biological sample obtained from a subject. For example, nucleic acid can be isolated from blood, saliva, sputum, urine, cell scrapings, and biopsy tissue. The nucleic acid sample can be isolated from a biological sample using standard techniques, such as the technique described in Example 2. As used herein, the term "subject" refers primarily to humans but also refers to other mammals such as dogs, cats, and ungulates (e.g., cattle, sheep, and swine). Subjects also include avians (e.g., chickens and turkeys), reptiles, and fish (e.g., salmon), as embodiments described herein can be adapted to nucleic acid samples isolated from any of these organisms. The nucleic acid sample may be isolated from the subject and then directly utilized in a

method for determining the presence of a polymorphic variant, or alternatively, the sample may be isolated and then stored (*e.g.*, frozen) for a period of time before being subjected to analysis.

**[0089]** The presence or absence of a polymorphic variant is determined using one or both chromosomal complements represented in the nucleic acid sample. Determining the presence or absence of a polymorphic variant in both chromosomal complements represented in a nucleic acid sample from a subject having a copy of each chromosome is useful for determining the zygosity of an individual for the polymorphic variant (*i.e.*, whether the individual is homozygous or heterozygous for the polymorphic variant). Any oligonucleotide-based diagnostic may be utilized to determine whether a sample includes the presence or absence of a polymorphic variant in a sample. For example, primer extension methods, ligase sequence determination methods (*e.g.*, U.S. Pat. Nos. 5,679,524 and 5,952,174, and WO 01/27326), mismatch sequence determination methods (*e.g.*, U.S. Pat. Nos. 5,851,770; 5,958,692; 6,110,684; and 6,183,958), microarray sequence determination methods, restriction fragment length polymorphism (RFLP), single strand conformation polymorphism detection (SSCP) (*e.g.*, U.S. Pat. Nos. 5,891,625 and 6,013,499), PCR-based assays (*e.g.*, TAQMAN<sup>®</sup> PCR System (Applied Biosystems)), and nucleotide sequencing methods may be used.

**[0090]** Oligonucleotide extension methods typically involve providing a pair of oligonucleotide primers in a polymerase chain reaction (PCR) or in other nucleic acid amplification methods for the purpose of amplifying a region from the nucleic acid sample that comprises the polymorphic variation. One oligonucleotide primer is complementary to a region 3' of the polymorphism and the other is complementary to a region 5' of the polymorphism. A PCR primer pair may be used in methods disclosed in U.S. Pat. Nos. 4,683,195; 4,683,202, 4,965,188; 5,656,493; 5,998,143; 6,140,054; WO 01/27327; and WO 01/27329 for example. PCR primer pairs may also be used in any commercially available machines that perform PCR, such as any of the GENEAMP<sup>®</sup> Systems available from Applied Biosystems. Also, those of ordinary skill in the art will be able to design oligonucleotide primers based upon a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence using knowledge available in the art.

**[0091]** Also provided is an extension oligonucleotide that hybridizes to the amplified fragment adjacent to the polymorphic variation. As used herein, the term "adjacent" refers to the 3' end of the extension oligonucleotide being often 1 nucleotide from the 5' end of the polymorphic site, and sometimes 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides from the 5' end of the polymorphic site, in the nucleic acid when the extension oligonucleotide is hybridized to the nucleic acid. The extension oligonucleotide then is extended by one or more nucleotides, and the number and/or type of nucleotides that are added to the extension oligonucleotide determine whether the polymorphic variant is present. Oligonucleotide extension methods are disclosed, for example, in U.S. Pat. Nos. 4,656,127; 4,851,331; 5,679,524; 5,834,189; 5,876,934; 5,908,755; 5,912,118; 5,976,802; 5,981,186; 6,004,744; 6,013,431; 6,017,702;

6,046,005; 6,087,095; 6,210,891; and WO 01/20039. Oligonucleotide extension methods using mass spectrometry are described, for example, in U.S. Pat. Nos. 5,547,835; 5,605,798; 5,691,141; 5,849,542; 5,869,242; 5,928,906; 6,043,031; and 6,194,144, and a method often utilized is described herein in Example 2.

**[0092]** A microarray can be utilized for determining whether a polymorphic variant is present or absent in a nucleic acid sample. A microarray may include any oligonucleotides described herein, and methods for making and using oligonucleotide microarrays suitable for diagnostic use are disclosed in U.S. Pat. Nos. 5,492,806; 5,525,464; 5,589,330; 5,695,940; 5,849,483; 6,018,041; 6,045,996; 6,136,541; 6,142,681; 6,156,501; 6,197,506; 6,223,127; 6,225,625; 6,229,911; 6,239,273; WO 00/52625; WO 01/25485; and WO 01/29259. The microarray typically comprises a solid support and the oligonucleotides may be linked to this solid support by covalent bonds or by non-covalent interactions. The oligonucleotides may also be linked to the solid support directly or by a spacer molecule. A microarray may comprise one or more oligonucleotides complementary to a polymorphic site set forth herein.

**[0093]** A kit also may be utilized for determining whether a polymorphic variant is present or absent in a nucleic acid sample. A kit often comprises one or more pairs of oligonucleotide primers useful for amplifying a fragment of a nucleotide sequence of SEQ ID NO: 1-12 or a substantially identical sequence thereof, where the fragment includes a polymorphic site. The kit sometimes comprises a polymerizing agent, for example, a thermostable nucleic acid polymerase such as one disclosed in U.S. Pat. Nos. 4,889,818 or 6,077,664. Also, the kit often comprises an elongation oligonucleotide that hybridizes to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence in a nucleic acid sample adjacent to the polymorphic site. Where the kit includes an elongation oligonucleotide, it also often comprises chain elongating nucleotides, such as dATP, dTTP, dGTP, dCTP, and dTTP, including analogs of dATP, dTTP, dGTP, dCTP and dTTP, provided that such analogs are substrates for a thermostable nucleic acid polymerase and can be incorporated into a nucleic acid chain elongated from the extension oligonucleotide. Along with chain elongating nucleotides would be one or more chain terminating nucleotides such as ddATP, ddTTP, ddGTP, ddCTP, and the like. In an embodiment, the kit comprises one or more oligonucleotide primer pairs, a polymerizing agent, chain elongating nucleotides, at least one elongation oligonucleotide, and one or more chain terminating nucleotides. Kits optionally include buffers, vials, microtiter plates, and instructions for use.

**[0094]** An individual identified as being at risk of osteoarthritis may be heterozygous or homozygous with respect to the allele associated with a higher risk of osteoarthritis. A subject homozygous for an allele associated with an increased risk of osteoarthritis is at a comparatively high risk of osteoarthritis, a subject heterozygous for an allele associated with an increased risk of osteoarthritis is at a comparatively intermediate risk of osteoarthritis, and a subject homozygous for an

allele associated with a decreased risk of osteoarthritis is at a comparatively low risk of osteoarthritis. A genotype may be assessed for a complementary strand, such that the complementary nucleotide at a particular position is detected.

[0095] Also featured are methods for determining risk of osteoarthritis and/or identifying a subject at risk of osteoarthritis by contacting a polypeptide or protein encoded by a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence from a subject with an antibody that specifically binds to an epitope associated with increased risk of osteoarthritis in the polypeptide.

#### Applications of Prognostic and Diagnostic Results to Pharmacogenomic Methods

[0096] Pharmacogenomics is a discipline that involves tailoring a treatment for a subject according to the subject's genotype as a particular treatment regimen may exert a differential effect depending upon the subject's genotype. For example, based upon the outcome of a prognostic test described herein, a clinician or physician may target pertinent information and preventative or therapeutic treatments to a subject who would be benefited by the information or treatment and avoid directing such information and treatments to a subject who would not be benefited (*e.g.*, the treatment has no therapeutic effect and/or the subject experiences adverse side effects).

[0097] The following is an example of a pharmacogenomic embodiment. A particular treatment regimen can exert a differential effect depending upon the subject's genotype. Where a candidate therapeutic exhibits a significant interaction with a major allele and a comparatively weak interaction with a minor allele (*e.g.*, an order of magnitude or greater difference in the interaction), such a therapeutic typically would not be administered to a subject genotyped as being homozygous for the minor allele, and sometimes not administered to a subject genotyped as being heterozygous for the minor allele. In another example, where a candidate therapeutic is not significantly toxic when administered to subjects who are homozygous for a major allele but is comparatively toxic when administered to subjects heterozygous or homozygous for a minor allele, the candidate therapeutic is not typically administered to subjects who are genotyped as being heterozygous or homozygous with respect to the minor allele.

[0098] The methods described herein are applicable to pharmacogenomic methods for preventing, alleviating or treating osteoarthritis. For example, a nucleic acid sample from an individual may be subjected to a prognostic test described herein. Where one or more polymorphic variations associated with increased risk of osteoarthritis are identified in a subject, information for preventing or treating osteoarthritis and/or one or more osteoarthritis treatment regimens then may be prescribed to that subject.

[0099] In certain embodiments, a treatment or preventative regimen is specifically prescribed and/or administered to individuals who will most benefit from it based upon their risk of developing osteoarthritis assessed by the methods described herein. Thus, provided are methods for identifying a subject predisposed to osteoarthritis and then prescribing a therapeutic or preventative regimen to

individuals identified as having a predisposition. Thus, certain embodiments are directed to a method for reducing osteoarthritis in a subject, which comprises: detecting the presence or absence of a polymorphic variant associated with osteoarthritis in a nucleotide sequence in a nucleic acid sample from a subject, where the nucleotide sequence comprises a polynucleotide sequence selected from the group consisting of: (a) a nucleotide sequence of SEQ ID NO: 1-12; (b) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-12; (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-12, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO: 1-12; and (d) a fragment of a polynucleotide sequence of (a), (b), or (c); and prescribing or administering a treatment regimen to a subject from whom the sample originated where the presence of a polymorphic variation associated with osteoarthritis is detected in the nucleotide sequence. In these methods, predisposition results may be utilized in combination with other test results to diagnose osteoarthritis.

**[0100]** Certain preventative treatments often are prescribed to subjects having a predisposition to osteoarthritis and where the subject is diagnosed with osteoarthritis or is diagnosed as having symptoms indicative of an early stage of osteoarthritis. The treatment sometimes is preventative (*e.g.*, is prescribed or administered to reduce the probability that osteoarthritis arises or progresses), sometimes is therapeutic, and sometimes delays, alleviates or halts the progression of osteoarthritis. Any known preventative or therapeutic treatment for alleviating or preventing the occurrence of osteoarthritis is prescribed and/or administered. For example, the treatment often is directed to decreasing pain and improving joint movement. Examples of OA treatments include exercises to keep joints flexible and improve muscle strength. Different medications to control pain, including corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs, *e.g.*, Voltaren); cyclooxygenase-2 (COX-2) inhibitors (*e.g.*, Celebrex, Vioxx, Mobic, and Bextra); monoclonal antibodies (*e.g.*, Remicade); tumor necrosis factor inhibitors (*e.g.*, Enbrel); or injections of glucocorticoids, hyaluronic acid or chondroitin sulfate into joints that are inflamed and not responsive to NSAIDs. Orally administered chondroitin sulfate also may be used as a therapeutic, as it may increase hyaluronic acid levels and viscosity of synovial fluid, and decrease collagenase levels in synovial fluid. Also, glucosamine can serve as an OA therapeutic as delivering it into joints may inhibit enzymes involved in cartilage degradation and enhance the production of hyaluronic acid. For mild pain without inflammation, acetaminophen may be used. Other treatments include: heat/cold therapy for temporary pain relief; joint protection to prevent strain or stress on painful joints; surgery to relieve chronic pain in damaged joints; and weight control to prevent extra stress on weight-bearing joints.

**[0101]** As therapeutic approaches for treating osteoarthritis continue to evolve and improve, the goal of treatments for osteoarthritis related disorders is to intervene even before clinical signs first manifest.

Thus, genetic markers associated with susceptibility to osteoarthritis prove useful for early diagnosis, prevention and treatment of osteoarthritis.

[0102] As osteoarthritis preventative and treatment information can be specifically targeted to subjects in need thereof (*e.g.*, those at risk of developing osteoarthritis or those in an early stage of osteoarthritis), provided herein is a method for preventing or reducing the risk of developing osteoarthritis in a subject, which comprises: (a) detecting the presence or absence of a polymorphic variation associated with osteoarthritis at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject; (b) identifying a subject with a predisposition to osteoarthritis, whereby the presence of the polymorphic variation is indicative of a predisposition to osteoarthritis in the subject; and (c) if such a predisposition is identified, providing the subject with information about methods or products to prevent or reduce osteoarthritis or to delay the onset of osteoarthritis. Also provided is a method of targeting information or advertising to a subpopulation of a human population based on the subpopulation being genetically predisposed to a disease or condition, which comprises: (a) detecting the presence or absence of a polymorphic variation associated with osteoarthritis at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject; (b) identifying the subpopulation of subjects in which the polymorphic variation is associated with osteoarthritis; and (c) providing information only to the subpopulation of subjects about a particular product which may be obtained and consumed or applied by the subject to help prevent or delay onset of the disease or condition.

[0103] Pharmacogenomics methods also may be used to analyze and predict a response to osteoarthritis treatment or a drug. For example, if pharmacogenomics analysis indicates a likelihood that an individual will respond positively to osteoarthritis treatment with a particular drug, the drug may be administered to the individual. Conversely, if the analysis indicates that an individual is likely to respond negatively to treatment with a particular drug, an alternative course of treatment may be prescribed. A negative response may be defined as either the absence of an efficacious response or the presence of toxic side effects. The response to a therapeutic treatment can be predicted in a background study in which subjects in any of the following populations are genotyped: a population that responds favorably to a treatment regimen, a population that does not respond significantly to a treatment regimen, and a population that responds adversely to a treatment regimen (*e.g.*, exhibits one or more side effects). These populations are provided as examples and other populations and subpopulations may be analyzed. Based upon the results of these analyses, a subject is genotyped to predict whether he or she will respond favorably to a treatment regimen, not respond significantly to a treatment regimen, or respond adversely to a treatment regimen.

[0104] The tests described herein also are applicable to clinical drug trials. One or more polymorphic variants indicative of response to an agent for treating osteoarthritis or to side effects to an agent for treating osteoarthritis may be identified using the methods described herein. Thereafter,

potential participants in clinical trials of such an agent may be screened to identify those individuals most likely to respond favorably to the drug and exclude those likely to experience side effects. In that way, the effectiveness of drug treatment may be measured in individuals who respond positively to the drug, without lowering the measurement as a result of the inclusion of individuals who are unlikely to respond positively in the study and without risking undesirable safety problems.

**[0105]** Thus, another embodiment is a method of selecting an individual for inclusion in a clinical trial of a treatment or drug comprising the steps of: (a) obtaining a nucleic acid sample from an individual; (b) determining the identity of a polymorphic variation which is associated with a positive response to the treatment or the drug, or at least one polymorphic variation which is associated with a negative response to the treatment or the drug in the nucleic acid sample, and (c) including the individual in the clinical trial if the nucleic acid sample contains said polymorphic variation associated with a positive response to the treatment or the drug or if the nucleic acid sample lacks said polymorphic variation associated with a negative response to the treatment or the drug. In addition, the methods described herein for selecting an individual for inclusion in a clinical trial of a treatment or drug encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination. The polymorphic variation may be in a sequence selected individually or in any combination from the group consisting of (i) a nucleotide sequence of SEQ ID NO: 1-12; (ii) a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-12; (iii) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-12, or a nucleotide sequence about 90% or more identical to a nucleotide sequence of SEQ ID NO: 1-12; and (iv) a fragment of a polynucleotide sequence of (i), (ii), or (iii) comprising the polymorphic site. The including step (c) optionally comprises administering the drug or the treatment to the individual if the nucleic acid sample contains the polymorphic variation associated with a positive response to the treatment or the drug and the nucleic acid sample lacks said biallelic marker associated with a negative response to the treatment or the drug.

**[0106]** Also provided herein is a method of partnering between a diagnostic/prognostic testing provider and a provider of a consumable product, which comprises: (a) the diagnostic/prognostic testing provider detects the presence or absence of a polymorphic variation associated with osteoarthritis at a polymorphic site in a nucleotide sequence in a nucleic acid sample from a subject; (b) the diagnostic/prognostic testing provider identifies the subpopulation of subjects in which the polymorphic variation is associated with osteoarthritis; (c) the diagnostic/prognostic testing provider forwards information to the subpopulation of subjects about a particular product which may be obtained and consumed or applied by the subject to help prevent or delay onset of the disease or condition; and (d) the

provider of a consumable product forwards to the diagnostic test provider a fee every time the diagnostic/prognostic test provider forwards information to the subject as set forth in step (c) above.

#### Compositions Comprising Osteoarthritis-Directed Molecules

**[0107]** Featured herein is a composition comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and one or more molecules specifically directed and targeted to a nucleic acid comprising a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence or amino acid sequence. Such directed molecules include, but are not limited to, a compound that binds to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence or amino acid sequence referenced herein; a RNAi or siRNA molecule having a strand complementary or substantially complementary to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence (e.g., hybridizes to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence under conditions of high stringency); an antisense nucleic acid complementary or substantially complementary to an RNA encoded by a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence (e.g., hybridizes to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence under conditions of high stringency); a ribozyme that hybridizes to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence (e.g., hybridizes to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence under conditions of high stringency); a nucleic acid aptamer that specifically binds a polypeptide encoded by *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence; and an antibody that specifically binds to a polypeptide encoded by *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence or binds to a nucleic acid having such a nucleotide sequence. In specific embodiments, the osteoarthritis directed molecule interacts with a nucleic acid or polypeptide variant associated with osteoarthritis, such as variants referenced herein. In other embodiments, the osteoarthritis directed molecule interacts with a polypeptide involved in a signal pathway of a polypeptide encoded by a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence, or a nucleic acid comprising such a nucleotide sequence.

**[0108]** Compositions sometimes include an adjuvant known to stimulate an immune response, and in certain embodiments, an adjuvant that stimulates a T-cell lymphocyte response. Adjuvants are known, including but not limited to an aluminum adjuvant (e.g., aluminum hydroxide); a cytokine adjuvant or adjuvant that stimulates a cytokine response (e.g., interleukin (IL)-12 and/or gamma-interferon cytokines); a Freund-type mineral oil adjuvant emulsion (e.g., Freund's complete or incomplete adjuvant); a synthetic lipid compound; a copolymer adjuvant (e.g., TitreMax); a saponin; Quil A; a liposome; an oil-in-water emulsion (e.g., an emulsion stabilized by Tween 80 and pluronic polyoxyethylene/polyoxypropylene block copolymer (Syntex Adjuvant Formulation); TitreMax; detoxified endotoxin (MPL) and mycobacterial cell wall components (TDW, CWS) in 2% squalene (Ribi Adjuvant System)); a muramyl dipeptide; an immune-stimulating complex (ISCOM, e.g., an Ag-

modified saponin/cholesterol micelle that forms stable cage-like structure); an aqueous phase adjuvant that does not have a depot effect (e.g., Gerbu adjuvant); a carbohydrate polymer (e.g., AdjuPrime); L-tyrosine; a manide-oleate compound (e.g., Montanide); an ethylene-vinyl acetate copolymer (e.g., Elvax 40W1,2); or lipid A, for example. Such compositions are useful for generating an immune response against osteoarthritis directed molecule (e.g., an HLA-binding subsequence within a polypeptide encoded by a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence). In such methods, a peptide having an amino acid subsequence of a polypeptide encoded by a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence is delivered to a subject, where the subsequence binds to an HLA molecule and induces a CTL lymphocyte response. The peptide sometimes is delivered to the subject as an isolated peptide or as a minigene in a plasmid that encodes the peptide. Methods for identifying HLA-binding subsequences in such polypeptides are known (see e.g., publication WO02/20616 and PCT application US98/01373 for methods of identifying such sequences).

[0109] The cell may be in a group of cells cultured *in vitro* or in a tissue maintained *in vitro* or present in an animal *in vivo* (e.g., a rat, mouse, ape or human). In certain embodiments, a composition comprises a component from a cell such as a nucleic acid molecule (e.g., genomic DNA), a protein mixture or isolated protein, for example. The aforementioned compositions have utility in diagnostic, prognostic and pharmacogenomic methods described previously and in therapeutics described hereafter. Certain osteoarthritis directed molecules are described in greater detail below.

### Compounds

[0110] Compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive (see, e.g., Zuckermann et al., J. Med. Chem. 37: 2678-85 (1994)); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; "one-bead one-compound" library methods; and synthetic library methods using affinity chromatography selection. Biological library and peptoid library approaches are typically limited to peptide libraries, while the other approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, Anticancer Drug Des. 12: 145, (1997)). Examples of methods for synthesizing molecular libraries are described, for example, in DeWitt et al., Proc. Natl. Acad. Sci. U.S.A. 90: 6909 (1993); Erb et al., Proc. Natl. Acad. Sci. USA 91: 11422 (1994); Zuckermann et al., J. Med. Chem. 37: 2678 (1994); Cho et al., Science 261: 1303 (1993); Carrell et al., Angew. Chem. Int. Ed. Engl. 33: 2059 (1994); Carell et al., Angew. Chem. Int. Ed. Engl. 33: 2061 (1994); and in Gallop et al., J. Med. Chem. 37: 1233 (1994).

[0111] Libraries of compounds may be presented in solution (e.g., Houghten, *Biotechniques* 13: 412-421 (1992)), or on beads (Lam, *Nature* 354: 82-84 (1991)), chips (Fodor, *Nature* 364: 555-556 (1993)), bacteria or spores (Ladner, United States Patent No. 5,223,409), plasmids (Cull et al., *Proc. Natl. Acad. Sci. USA* 89: 1865-1869 (1992)) or on phage (Scott and Smith, *Science* 249: 386-390 (1990); Devlin, *Science* 249: 404-406 (1990); Cwirla et al., *Proc. Natl. Acad. Sci.* 87: 6378-6382 (1990); Felici, *J. Mol. Biol.* 222: 301-310 (1991); Ladner supra.).

[0112] A compound sometimes alters expression and sometimes alters activity of a polypeptide target and may be a small molecule. Small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

#### Antisense Nucleic Acid Molecules, Ribozymes, RNAi, siRNA and Modified Nucleic Acid Molecules

[0113] An “antisense” nucleic acid refers to a nucleotide sequence complementary to a “sense” nucleic acid encoding a polypeptide, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire coding strand, or to a portion thereof or a substantially identical sequence thereof. In another embodiment, the antisense nucleic acid molecule is antisense to a “noncoding region” of the coding strand of a nucleotide sequence (e.g., 5’ and 3’ untranslated regions in SEQ ID NO: 1).

[0114] An antisense nucleic acid can be designed such that it is complementary to the entire coding region of an mRNA encoded by a nucleotide sequence (e.g., SEQ ID NO: 1), and often the antisense nucleic acid is an oligonucleotide antisense to only a portion of a coding or noncoding region of the mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of the mRNA, e.g., between the -10 and +10 regions of the target gene nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length. The antisense nucleic acids, which include the ribozymes described hereafter, can be designed to target a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence, often a variant associated with osteoarthritis, or a substantially identical sequence thereof. Among the variants, minor alleles and major alleles can be targeted, and

those associated with a higher risk of osteoarthritis are often designed, tested, and administered to subjects.

**[0115]** An antisense nucleic acid can be constructed using chemical synthesis and enzymatic ligation reactions using standard procedures. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

**[0116]** When utilized as therapeutics, antisense nucleic acids typically are administered to a subject (e.g., by direct injection at a tissue site) or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a polypeptide and thereby inhibit expression of the polypeptide, for example, by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then are administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, for example, by linking antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. Antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. Sufficient intracellular concentrations of antisense molecules are achieved by incorporating a strong promoter, such as a pol II or pol III promoter, in the vector construct.

**[0117]** Antisense nucleic acid molecules sometimes are alpha-anomeric nucleic acid molecules. An alpha-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual beta-units, the strands run parallel to each other (Gaultier et al., Nucleic Acids. Res. 15: 6625-6641 (1987)). Antisense nucleic acid molecules can also comprise a 2'-o-methylribonucleotide (Inoue et al., Nucleic Acids Res. 15: 6131-6148 (1987)) or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215: 327-330 (1987)). Antisense nucleic acids sometimes are composed of DNA or PNA or any other nucleic acid derivatives described previously.

**[0118]** In another embodiment, an antisense nucleic acid is a ribozyme. A ribozyme having specificity for a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence can include one or more sequences complementary to such a nucleotide sequence, and a sequence having a known catalytic region responsible for mRNA cleavage (see e.g., U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach, Nature 334: 585-591 (1988)). For example, a derivative of a Tetrahymena L-19 IVS RNA is sometimes utilized in which the nucleotide sequence of the active site is complementary to the nucleotide sequence

to be cleaved in a mRNA (see e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742). Also, target mRNA sequences can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (see e.g., Bartel & Szostak, Science 261: 1411-1418 (1993)).

**[0119]** Osteoarthritis directed molecules include in certain embodiments nucleic acids that can form triple helix structures with a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence, or a substantially identical sequence thereof, especially one that includes a regulatory region that controls expression of a polypeptide. Gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of a nucleotide sequence referenced herein or a substantially identical sequence (e.g., promoter and/or enhancers) to form triple helical structures that prevent transcription of a gene in target cells (see e.g., Helene, Anticancer Drug Des. 6(6): 569-84 (1991); Helene et al., Ann. N.Y. Acad. Sci. 660: 27-36 (1992); and Maher, Bioassays 14(12): 807-15 (1992). Potential sequences that can be targeted for triple helix formation can be increased by creating a so-called “switchback” nucleic acid molecule. Switchback molecules are synthesized in an alternating 5’-3’, 3’-5’ manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

**[0120]** Osteoarthritis directed molecules include RNAi and siRNA nucleic acids. Gene expression may be inhibited by the introduction of double-stranded RNA (dsRNA), which induces potent and specific gene silencing, a phenomenon called RNA interference or RNAi. See, e.g., Fire et al., US Patent Number 6,506,559; Tuschl et al. PCT International Publication No. WO 01/75164; Kay et al. PCT International Publication No. WO 03/010180A1; or Boshier JM, Labouesse, Nat Cell Biol 2000 Feb;2(2):E31-6. This process has been improved by decreasing the size of the double-stranded RNA to 20-24 base pairs (to create small-interfering RNAs or siRNAs) that “switched off” genes in mammalian cells without initiating an acute phase response, i.e., a host defense mechanism that often results in cell death (see, e.g., Caplen et al. Proc Natl Acad Sci U S A. 2001 Aug 14;98(17):9742-7 and Elbashir et al. Methods 2002 Feb;26(2):199-213). There is increasing evidence of post-transcriptional gene silencing by RNA interference (RNAi) for inhibiting targeted expression in mammalian cells at the mRNA level, in human cells. There is additional evidence of effective methods for inhibiting the proliferation and migration of tumor cells in human patients, and for inhibiting metastatic cancer development (see, e.g., U.S. Patent Application No. US2001000993183; Caplen et al. Proc Natl Acad Sci U S A; and Abderrahmani et al. Mol Cell Biol 2001 Nov21(21):7256-67).

**[0121]** An “siRNA” or “RNAi” refers to a nucleic acid that forms a double stranded RNA and has the ability to reduce or inhibit expression of a gene or target gene when the siRNA is delivered to or expressed in the same cell as the gene or target gene. “siRNA” refers to short double-stranded RNA formed by the complementary strands. Complementary portions of the siRNA that hybridize to form the

double stranded molecule often have substantial or complete identity to the target molecule sequence. In one embodiment, an siRNA refers to a nucleic acid that has substantial or complete identity to a target gene and forms a double stranded siRNA.

**[0122]** When designing the siRNA molecules, the targeted region often is selected from a given DNA sequence beginning 50 to 100 nucleotides downstream of the start codon. See, e.g., Elbashir et al., *Methods* 26:199-213 (2002). Initially, 5' or 3' UTRs and regions nearby the start codon were avoided assuming that UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNP or RISC endonuclease complex. Sometimes regions of the target 23 nucleotides in length conforming to the sequence motif AA(N19)TT (N, an nucleotide), and regions with approximately 30% to 70% G/C-content (often about 50% G/C-content) often are selected. If no suitable sequences are found, the search often is extended using the motif NA(N21). The sequence of the sense siRNA sometimes corresponds to (N19) TT or N21 (position 3 to 23 of the 23-nt motif), respectively. In the latter case, the 3' end of the sense siRNA often is converted to TT. The rationale for this sequence conversion is to generate a symmetric duplex with respect to the sequence composition of the sense and antisense 3' overhangs. The antisense siRNA is synthesized as the complement to position 1 to 21 of the 23-nt motif. Because position 1 of the 23-nt motif is not recognized sequence-specifically by the antisense siRNA, the 3'-most nucleotide residue of the antisense siRNA can be chosen deliberately. However, the penultimate nucleotide of the antisense siRNA (complementary to position 2 of the 23-nt motif) often is complementary to the targeted sequence. For simplifying chemical synthesis, TT often is utilized. siRNAs corresponding to the target motif NAR(N17)YNN, where R is purine (A,G) and Y is pyrimidine (C,U), often are selected. Respective 21 nucleotide sense and antisense siRNAs often begin with a purine nucleotide and can also be expressed from pol III expression vectors without a change in targeting site. Expression of RNAs from pol III promoters often is efficient when the first transcribed nucleotide is a purine.

**[0123]** The sequence of the siRNA can correspond to the full length target gene, or a subsequence thereof. Often, the siRNA is about 15 to about 50 nucleotides in length (e.g., each complementary sequence of the double stranded siRNA is 15-50 nucleotides in length, and the double stranded siRNA is about 15-50 base pairs in length, sometimes about 20-30 nucleotides in length or about 20-25 nucleotides in length, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. The siRNA sometimes is about 21 nucleotides in length. Methods of using siRNA are well known in the art, and specific siRNA molecules may be purchased from a number of companies including Dharmacon Research, Inc.

**[0124]** Antisense, ribozyme, RNAi and siRNA nucleic acids can be altered to form modified nucleic acid molecules. The nucleic acids can be altered at base moieties, sugar moieties or phosphate backbone moieties to improve stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of nucleic acid molecules can be modified to generate peptide nucleic acids (see

Hyrup et al., *Bioorganic & Medicinal Chemistry* 4 (1): 5-23 (1996)). As used herein, the terms “peptide nucleic acid” or “PNA” refers to a nucleic acid mimic such as a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. Synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described, for example, in Hyrup et al., (1996) *supra* and Perry-O’Keefe et al., *Proc. Natl. Acad. Sci.* 93: 14670-675 (1996).

[0125] PNA nucleic acids can be used in prognostic, diagnostic, and therapeutic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNA nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as “artificial restriction enzymes” when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup et al., (1996) *supra*; Perry-O’Keefe *supra*).

[0126] In other embodiments, oligonucleotides may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across cell membranes (see e.g., Letsinger et al., *Proc. Natl. Acad. Sci. USA* 86: 6553-6556 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci. USA* 84: 648-652 (1987); PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al., *Bio-Techniques* 6: 958-976 (1988)) or intercalating agents. (See, e.g., Zon, *Pharm. Res.* 5: 539-549 (1988) ). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

[0127] Also included herein are molecular beacon oligonucleotide primer and probe molecules having one or more regions complementary to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence, or a substantially identical sequence thereof, two complementary regions one having a fluorophore and one a quencher such that the molecular beacon is useful for quantifying the presence of the nucleic acid in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi et al., U.S. Patent No. 5,854,033; Nazarenko et al., U.S. Patent No. 5,866,336, and Livak et al., U.S. Patent 5,876,930.

### Antibodies

[0128] The term “antibody” as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab’)<sub>2</sub> fragments which can be

generated by treating the antibody with an enzyme such as pepsin. An antibody sometimes is a polyclonal, monoclonal, recombinant (e.g., a chimeric or humanized), fully human, non-human (e.g., murine), or a single chain antibody. An antibody may have effector function and can fix complement, and is sometimes coupled to a toxin or imaging agent.

**[0129]** A full-length polypeptide or antigenic peptide fragment encoded by a nucleotide sequence referenced herein can be used as an immunogen or can be used to identify antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. An antigenic peptide often includes at least 8 amino acid residues of the amino acid sequences encoded by a nucleotide sequence referenced herein, or substantially identical sequence thereof, and encompasses an epitope. Antigenic peptides sometimes include 10 or more amino acids, 15 or more amino acids, 20 or more amino acids, or 30 or more amino acids. Hydrophilic and hydrophobic fragments of polypeptides sometimes are used as immunogens.

**[0130]** Epitopes encompassed by the antigenic peptide are regions located on the surface of the polypeptide (e.g., hydrophilic regions) as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human polypeptide sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the polypeptide and are thus likely to constitute surface residues useful for targeting antibody production. The antibody may bind an epitope on any domain or region on polypeptides described herein.

**[0131]** Also, chimeric, humanized, and completely human antibodies are useful for applications which include repeated administration to subjects. Chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, can be made using standard recombinant DNA techniques. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al International Application No. PCT/US86/02269; Akira, et al European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al European Patent Application 173,494; Neuberger et al PCT International Publication No. WO 86/01533; Cabilly et al U.S. Patent No. 4,816,567; Cabilly et al European Patent Application 125,023; Better et al., Science 240: 1041-1043 (1988); Liu et al., Proc. Natl. Acad. Sci. USA 84: 3439-3443 (1987); Liu et al., J. Immunol. 139: 3521-3526 (1987); Sun et al., Proc. Natl. Acad. Sci. USA 84: 214-218 (1987); Nishimura et al., Canc. Res. 47: 999-1005 (1987); Wood et al., Nature 314: 446-449 (1985); and Shaw et al., J. Natl. Cancer Inst. 80: 1553-1559 (1988); Morrison, S. L., Science 229: 1202-1207 (1985); Oi et al., BioTechniques 4: 214 (1986); Winter U.S. Patent 5,225,539; Jones et al., Nature 321: 552-525 (1986); Verhoeyan et al., Science 239: 1534; and Beidler et al., J. Immunol. 141: 4053-4060 (1988).

**[0132]** Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice that are incapable of expressing

endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. See, for example, Lonberg and Huszar, *Int. Rev. Immunol.* 13: 65-93 (1995); and U.S. Patent Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806. In addition, companies such as Abgenix, Inc. (Fremont, CA) and Medarex, Inc. (Princeton, NJ), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above. Completely human antibodies that recognize a selected epitope also can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody (e.g., a murine antibody) is used to guide the selection of a completely human antibody recognizing the same epitope. This technology is described for example by Jespers et al., *Bio/Technology* 12: 899-903 (1994).

**[0133]** An antibody can be a single chain antibody. A single chain antibody (scFV) can be engineered (see, e.g., Colcher et al., *Ann. N Y Acad. Sci.* 880: 263-80 (1999); and Reiter, *Clin. Cancer Res.* 2: 245-52 (1996)). Single chain antibodies can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target polypeptide.

**[0134]** Antibodies also may be selected or modified so that they exhibit reduced or no ability to bind an Fc receptor. For example, an antibody may be an isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor (e.g., it has a mutagenized or deleted Fc receptor binding region).

**[0135]** Also, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1 dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thiotepa chlorambucil, melphalan, carmustine (BCNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

**[0136]** Antibody conjugates can be used for modifying a given biological response. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide such as tumor necrosis factor, gamma-interferon, alpha-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for

example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Also, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, for example.

**[0137]** An antibody (e.g., monoclonal antibody) can be used to isolate target polypeptides by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an antibody can be used to detect a target polypeptide (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. Antibodies can be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure, e.g., to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labeling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ . Also, an antibody can be utilized as a test molecule for determining whether it can treat osteoarthritis, and as a therapeutic for administration to a subject for treating osteoarthritis.

**[0138]** An antibody can be made by immunizing with a purified antigen, or a fragment thereof, e.g., a fragment described herein, a membrane associated antigen, tissues, e.g., crude tissue preparations, whole cells, preferably living cells, lysed cells, or cell fractions.

**[0139]** Included herein are antibodies which bind only a native polypeptide, only denatured or otherwise non-native polypeptide, or which bind both, as well as those having linear or conformational epitopes. Conformational epitopes sometimes can be identified by selecting antibodies that bind to native but not denatured polypeptide. Also featured are antibodies that specifically bind to a polypeptide variant associated with osteoarthritis.

#### Methods for Identifying Candidate Therapeutics for Treating Osteoarthritis

**[0140]** Current therapies for the treatment of osteoarthritis have limited efficacy, limited tolerability and significant mechanism-based side effects, and few of the available therapies adequately address underlying defects. Current therapeutic approaches were largely developed in the absence of defined molecular targets or even a solid understanding of disease pathogenesis. Therefore, provided are

methods of identifying candidate therapeutics that target biochemical pathways related to the development of osteoarthritis.

[0141] Thus, featured herein are methods for identifying a candidate therapeutic for treating osteoarthritis. The methods comprise contacting a test molecule with a target molecule in a system. A “target molecule” as used herein refers to a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid, a substantially identical nucleic acid thereof, or a fragment thereof, and an encoded polypeptide of the foregoing. The methods also comprise determining the presence or absence of an interaction between the test molecule and the target molecule, where the presence of an interaction between the test molecule and the nucleic acid or polypeptide identifies the test molecule as a candidate osteoarthritis therapeutic. The interaction between the test molecule and the target molecule may be quantified.

[0142] Test molecules and candidate therapeutics include, but are not limited to, compounds, antisense nucleic acids, siRNA molecules, ribozymes, polypeptides or proteins encoded by a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleotide sequence, or a substantially identical sequence or fragment thereof, and immunotherapeutics (e.g., antibodies and HLA-presented polypeptide fragments). A test molecule or candidate therapeutic may act as a modulator of target molecule concentration or target molecule function in a system. A “modulator” may agonize (i.e., up-regulates) or antagonize (i.e., down-regulates) a target molecule concentration partially or completely in a system by affecting such cellular functions as DNA replication and/or DNA processing (e.g., DNA methylation or DNA repair), RNA transcription and/or RNA processing (e.g., removal of intronic sequences and/or translocation of spliced mRNA from the nucleus), polypeptide production (e.g., translation of the polypeptide from mRNA), and/or polypeptide post-translational modification (e.g., glycosylation, phosphorylation, and proteolysis of pro-polypeptides). A modulator may also agonize or antagonize a biological function of a target molecule partially or completely, where the function may include adopting a certain structural conformation, interacting with one or more binding partners, ligand binding, catalysis (e.g., phosphorylation, dephosphorylation, hydrolysis, methylation, and isomerization), and an effect upon a cellular event (e.g., effecting progression of osteoarthritis).

[0143] As used herein, the term “system” refers to a cell free *in vitro* environment and a cell-based environment such as a collection of cells, a tissue, an organ, or an organism. A system is “contacted” with a test molecule in a variety of manners, including adding molecules in solution and allowing them to interact with one another by diffusion, cell injection, and any administration routes in an animal. As used herein, the term “interaction” refers to an effect of a test molecule on test molecule, where the effect sometimes is binding between the test molecule and the target molecule, and sometimes is an observable change in cells, tissue, or organism.

[0144] There are many standard methods for detecting the presence or absence of interaction between a test molecule and a target molecule. For example, titrametric, acidimetric, radiometric, NMR,

monolayer, polarographic, spectrophotometric, fluorescent, and ESR assays probative of a target molecule interaction may be utilized. Any modulator can be utilized in methods for detecting an interaction. For example, proteasome modulators (e.g., *PSMB1* includes a proteasome domain) are described in WO-2004014882 and Roesel et al. Proceedings of the American Association of Cancer Research 2003, 44:1st Ed (Abs 1769), and bortezomib (Velcade, MLN-341, LDP-341 and PS-341), a ubiquitin proteasome inhibitor, is used for the treatment of multiple myeloma.

**[0145]** Test molecule/target molecule interactions can be detected and/or quantified using assays known in the art. For example, an interaction can be determined by labeling the test molecule and/or the target molecule, where the label is covalently or non-covalently attached to the test molecule or target molecule. The label is sometimes a radioactive molecule such as  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ , which can be detected by direct counting of radioemission or by scintillation counting. Also, enzymatic labels such as horseradish peroxidase, alkaline phosphatase, or luciferase may be utilized where the enzymatic label can be detected by determining conversion of an appropriate substrate to product. In addition, presence or absence of an interaction can be determined without labeling. For example, a microphysiometer (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indication of an interaction between a test molecule and target molecule (McConnell, H. M. *et al.*, *Science* 257: 1906-1912 (1992)).

**[0146]** In cell-based systems, cells typically include a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid, an encoded polypeptide, or substantially identical nucleic acid or polypeptide thereof, and are often of mammalian origin, although the cell can be of any origin. Whole cells, cell homogenates, and cell fractions (e.g., cell membrane fractions) can be subjected to analysis. Where interactions between a test molecule with a target polypeptide are monitored, soluble and/or membrane bound forms of the polypeptide may be utilized. Where membrane-bound forms of the polypeptide are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate.

**[0147]** An interaction between a test molecule and target molecule also can be detected by monitoring fluorescence energy transfer (FET) (see, e.g., Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos *et al.* U.S. Patent No. 4,868,103). A fluorophore label on a first, "donor" molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second,

“acceptor” molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the “donor” polypeptide molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the “acceptor” molecule label may be differentiated from that of the “donor”. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the “acceptor” molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (*e.g.*, using a fluorimeter).

**[0148]** In another embodiment, determining the presence or absence of an interaction between a test molecule and a target molecule can be effected by monitoring surface plasmon resonance (*see, e.g.*, Sjolander & Urbanicz, *Anal. Chem.* 63: 2338-2345 (1991) and Szabo *et al.*, *Curr. Opin. Struct. Biol.* 5: 699-705 (1995)). “Surface plasmon resonance” or “biomolecular interaction analysis (BIA)” can be utilized to detect biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

**[0149]** In another embodiment, the target molecule or test molecules are anchored to a solid phase, facilitating the detection of target molecule/test molecule complexes and separation of the complexes from free, uncomplexed molecules. The target molecule or test molecule is immobilized to the solid support. In an embodiment, the target molecule is anchored to a solid surface, and the test molecule, which is not anchored, can be labeled, either directly or indirectly, with detectable labels discussed herein.

**[0150]** It may be desirable to immobilize a target molecule, an anti-target molecule antibody, and/or test molecules to facilitate separation of target molecule/test molecule complexes from uncomplexed forms, as well as to accommodate automation of the assay. The attachment between a test molecule and/or target molecule and the solid support may be covalent or non-covalent (*see, e.g.*, U.S. Patent No. 6,022,688 for non-covalent attachments). The solid support may be one or more surfaces of the system, such as one or more surfaces in each well of a microtiter plate, a surface of a silicon wafer, a surface of a bead (*see, e.g.*, Lam, *Nature* 354: 82-84 (1991)) that is optionally linked to another solid support, or a channel in a microfluidic device, for example. Types of solid supports, linker molecules for covalent and non-covalent attachments to solid supports, and methods for immobilizing nucleic acids and other molecules to solid supports are well known (*see, e.g.*, U.S. Patent Nos. 6,261,776; 5,900,481; 6,133,436; and 6,022,688; and WIPO publication WO 01/18234).

**[0151]** In an embodiment, target molecule may be immobilized to surfaces via biotin and streptavidin. For example, biotinylated target polypeptide can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). In another embodiment, a target polypeptide can be prepared as a fusion polypeptide. For example, glutathione-S-transferase/target polypeptide fusion can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivitized microtiter plates, which are then combined with a test molecule under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, or the matrix is immobilized in the case of beads, and complex formation is determined directly or indirectly as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of target molecule binding or activity is determined using standard techniques.

**[0152]** In an embodiment, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that a significant percentage of complexes formed will remain immobilized to the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of manners. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface, *e.g.*, by adding a labeled antibody specific for the immobilized component, where the antibody, in turn, can be directly labeled or indirectly labeled with, *e.g.*, a labeled anti-Ig antibody.

**[0153]** In another embodiment, an assay is performed utilizing antibodies that specifically bind target molecule or test molecule but do not interfere with binding of the target molecule to the test molecule. Such antibodies can be derivitized to a solid support, and unbound target molecule may be immobilized by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the target molecule.

**[0154]** Cell free assays also can be conducted in a liquid phase. In such an assay, reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (*see, e.g.*, Rivas, G., and Minton, *Trends Biochem Sci Aug;18(8):284-7 (1993)*); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (*see, e.g.*, Ausubel *et al.*, eds. *Current Protocols in Molecular Biology*, J. Wiley: New York (1999)); and immunoprecipitation (*see, e.g.*, Ausubel *et al.*, eds., *supra*). Media and

chromatographic techniques are known to one skilled in the art (*see, e.g., Heegaard, J Mol. Recognit. Winter; 11(1-6): 141-8 (1998); Hage & Tweed, J. Chromatogr. B Biomed. Sci. Appl. Oct 10; 699 (1-2): 499-525 (1997)*). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

**[0155]** In another embodiment, modulators of target molecule expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of target mRNA or target polypeptide is evaluated relative to the level of expression of target mRNA or target polypeptide in the absence of the candidate compound. When expression of target mRNA or target polypeptide is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as an agonist of target mRNA or target polypeptide expression. Alternatively, when expression of target mRNA or target polypeptide is less (*e.g., less with statistical significance*) in the presence of the candidate compound than in its absence, the candidate compound is identified as an antagonist or inhibitor of target mRNA or target polypeptide expression. The level of target mRNA or target polypeptide expression can be determined by methods described herein.

**[0156]** In another embodiment, binding partners that interact with a target molecule are detected. The target molecules can interact with one or more cellular or extracellular macromolecules, such as polypeptides *in vivo*, and these interacting molecules are referred to herein as “binding partners.” Binding partners can agonize or antagonize target molecule biological activity. Also, test molecules that agonize or antagonize interactions between target molecules and binding partners can be useful as therapeutic molecules as they can up-regulate or down-regulated target molecule activity *in vivo* and thereby treat osteoarthritis.

**[0157]** Binding partners of target molecules can be identified by methods known in the art. For example, binding partners may be identified by lysing cells and analyzing cell lysates by electrophoretic techniques. Alternatively, a two-hybrid assay or three-hybrid assay can be utilized (*see, e.g., U.S. Patent No. 5,283,317; Zervos et al., Cell 72:223-232 (1993); Madura et al., J. Biol. Chem. 268: 12046-12054 (1993); Bartel et al., Biotechniques 14: 920-924 (1993); Iwabuchi et al., Oncogene 8: 1693-1696 (1993); and Brent WO94/10300*). A two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. The assay often utilizes two different DNA constructs. In one construct, a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid (sometimes referred to as the “bait”) is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g., GAL-4*). In another construct, a DNA sequence from a library of DNA sequences that encodes a potential binding partner (sometimes referred to as the “prey”) is fused to a gene that encodes an activation domain of the known transcription factor. Sometimes, a *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1* or *ERG* nucleic acid can be fused to the activation domain. If the “bait” and the “prey” molecules interact *in vivo*, the DNA-binding and activation domains of the transcription factor are

brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to identify the potential binding partner.

**[0158]** In an embodiment for identifying test molecules that antagonize or agonize complex formation between target molecules and binding partners, a reaction mixture containing the target molecule and the binding partner is prepared, under conditions and for a time sufficient to allow complex formation. The reaction mixture often is provided in the presence or absence of the test molecule. The test molecule can be included initially in the reaction mixture, or can be added at a time subsequent to the addition of the target molecule and its binding partner. Control reaction mixtures are incubated without the test molecule or with a placebo. Formation of any complexes between the target molecule and the binding partner then is detected. Decreased formation of a complex in the reaction mixture containing test molecule as compared to in a control reaction mixture indicates that the molecule antagonizes target molecule/binding partner complex formation. Alternatively, increased formation of a complex in the reaction mixture containing test molecule as compared to in a control reaction mixture indicates that the molecule agonizes target molecule/binding partner complex formation. In another embodiment, complex formation of target molecule/binding partner can be compared to complex formation of mutant target molecule/binding partner (*e.g.*, amino acid modifications in a target polypeptide). Such a comparison can be important in those cases where it is desirable to identify test molecules that modulate interactions of mutant but not non-mutated target gene products.

**[0159]** The assays can be conducted in a heterogeneous or homogeneous format. In heterogeneous assays, target molecule and/or the binding partner are immobilized to a solid phase, and complexes are detected on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the molecules being tested. For example, test compounds that agonize target molecule/binding partner interactions can be identified by conducting the reaction in the presence of the test molecule in a competition format. Alternatively, test molecules that agonize preformed complexes, *e.g.*, molecules with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed.

**[0160]** In a heterogeneous assay embodiment, the target molecule or the binding partner is anchored onto a solid surface (*e.g.*, a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored molecule can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the molecule to be anchored can be used to anchor the molecule to the solid surface. The partner of the immobilized species is exposed to the coated surface with or without the test molecule. After the reaction is complete, unreacted components are removed

(*e.g.*, by washing) such that a significant portion of any complexes formed will remain immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface is indicative of complex. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored to the surface; *e.g.*, by using a labeled antibody specific for the initially non-immobilized species. Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

**[0161]** In another embodiment, the reaction can be conducted in a liquid phase in the presence or absence of test molecule, where the reaction products are separated from unreacted components, and the complexes are detected (*e.g.*, using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes). Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

**[0162]** In an alternate embodiment, a homogeneous assay can be utilized. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared. One or both of the target molecule or binding partner is labeled, and the signal generated by the label(s) is quenched upon complex formation (*e.g.*, U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). Addition of a test molecule that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target molecule/binding partner complexes can be identified.

**[0163]** Candidate therapeutics for treating osteoarthritis are identified from a group of test molecules that interact with a target molecule. Test molecules are normally ranked according to the degree with which they modulate (*e.g.*, agonize or antagonize) a function associated with the target molecule (*e.g.*, DNA replication and/or processing, RNA transcription and/or processing, polypeptide production and/or processing, and/or biological function/activity), and then top ranking modulators are selected. Also, pharmacogenomic information described herein can determine the rank of a modulator. The top 10% of ranked test molecules often are selected for further testing as candidate therapeutics, and sometimes the top 15%, 20%, or 25% of ranked test molecules are selected for further testing as candidate therapeutics. Candidate therapeutics typically are formulated for administration to a subject.

#### Therapeutic Formulations

**[0164]** Formulations and pharmaceutical compositions typically include in combination with a pharmaceutically acceptable carrier one or more target molecule modulators. The modulator often is a test molecule identified as having an interaction with a target molecule by a screening method described above. The modulator may be a compound, an antisense nucleic acid, a ribozyme, an antibody, or a

binding partner. Also, formulations may comprise a target polypeptide or fragment thereof in combination with a pharmaceutically acceptable carrier.

[0165] As used herein, the term “pharmaceutically acceptable carrier” includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions. Pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0166] A pharmaceutical composition typically is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0167] Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0168] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can

be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0169] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0170] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[0171] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art. Molecules can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0172] In one embodiment, active molecules are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to

viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[0173] It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[0174] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Molecules which exhibit high therapeutic indices are preferred. While molecules that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0175] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such molecules lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any molecules used in the methods described herein, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0176] As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, sometimes about 0.01 to 25 mg/kg body weight, often about 0.1 to 20 mg/kg body weight, and more often about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, sometimes between 2 to 8 weeks, often between about 3 to 7 weeks, and more often for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a

therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

[0177] With regard to polypeptide formulations, featured herein is a method for treating osteoarthritis in a subject, which comprises contacting one or more cells in the subject with a first polypeptide, where the subject comprises a second polypeptide having one or more polymorphic variations associated with cancer, and where the first polypeptide comprises fewer polymorphic variations associated with cancer than the second polypeptide. The first and second polypeptides are encoded by a nucleic acid which comprises a nucleotide sequence in SEQ ID NO: 1-12; a nucleotide sequence which encodes a polypeptide consisting of an amino acid sequence encoded by a nucleotide sequence referenced in SEQ ID NO: 1-12; a nucleotide sequence which encodes a polypeptide that is 90% or more identical to an amino acid sequence encoded by a nucleotide sequence of SEQ ID NO: 1-12 and a nucleotide sequence 90% or more identical to a nucleotide sequence in SEQ ID NO: 1-12. The subject often is a human.

[0178] For antibodies, a dosage of 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg) is often utilized. If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is often appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (*e.g.*, into the brain). A method for lipidation of antibodies is described by Cruikshank *et al.*, *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193 (1997).

[0179] Antibody conjugates can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a polypeptide such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

[0180] For compounds, exemplary doses include milligram or microgram amounts of the compound per kilogram of subject or sample weight, for example, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is understood that appropriate

doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid described herein, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

**[0181]** With regard to nucleic acid formulations, gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent 5,328,470) or by stereotactic injection (*see e.g.*, Chen *et al.*, (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). Pharmaceutical preparations of gene therapy vectors can include a gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells (*e.g.*, retroviral vectors) the pharmaceutical preparation can include one or more cells which produce the gene delivery system. Examples of gene delivery vectors are described herein.

#### Therapeutic Methods

**[0182]** A therapeutic formulation described above can be administered to a subject in need of a therapeutic for inducing a desired biological response. Therapeutic formulations can be administered by any of the paths described herein. With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from pharmacogenomic analyses described herein.

**[0183]** As used herein, the term “treatment” is defined as the application or administration of a therapeutic formulation to a subject, or application or administration of a therapeutic agent to an isolated tissue or cell line from a subject with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect osteoarthritis, symptoms of osteoarthritis or a predisposition towards osteoarthritis. A therapeutic formulation includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides. Administration of a therapeutic formulation can occur prior to the manifestation of symptoms characteristic of osteoarthritis, such that osteoarthritis is prevented or delayed in its progression. The appropriate therapeutic composition can be determined based on screening assays described herein.

**[0184]** As discussed, successful treatment of osteoarthritis can be brought about by techniques that serve to agonize target molecule expression or function, or alternatively, antagonize target molecule

expression or function. These techniques include administration of modulators that include, but are not limited to, small organic or inorganic molecules; antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, F(ab')<sub>2</sub> and Fab expression library fragments, scFV molecules, and epitope-binding fragments thereof); and peptides, phosphopeptides, or polypeptides.

**[0185]** Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above. It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular polypeptide, it can be preferable to co-administer normal target gene polypeptide into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

**[0186]** Another method by which nucleic acid molecules may be utilized in treating or preventing osteoarthritis is use of aptamer molecules specific for target molecules. Aptamers are nucleic acid molecules having a tertiary structure which permits them to specifically bind to ligands (*see, e.g., Osborne, et al., Curr. Opin. Chem. Biol.* 1(1): 5-9 (1997); and Patel, D. J., *Curr. Opin. Chem. Biol. Jun;1(1): 32-46 (1997)*).

**[0187]** Yet another method of utilizing nucleic acid molecules for osteoarthritis treatment is gene therapy, which can also be referred to as allele therapy. Provided herein is a gene therapy method for treating osteoarthritis in a subject, which comprises contacting one or more cells in the subject or from the subject with a nucleic acid having a first nucleotide sequence (e.g., the first nucleotide sequence is identical to or substantially identical to a nucleotide sequence of SEQ ID NO: 1-12). Genomic DNA in the subject comprises a second nucleotide sequence having one or more polymorphic variations associated with osteoarthritis (e.g., the second nucleotide sequence is identical to or substantially identical to a nucleotide sequence of SEQ ID NO: 1-4). The first and second nucleotide sequences typically are substantially identical to one another, and the first nucleotide sequence comprises fewer polymorphic variations associated with osteoarthritis than the second nucleotide sequence. The first nucleotide sequence may comprise a gene sequence that encodes a full-length polypeptide or a fragment thereof. The subject is often a human. Allele therapy methods often are utilized in conjunction with a

method of first determining whether a subject has genomic DNA that includes polymorphic variants associated with osteoarthritis.

**[0188]** In another allele therapy embodiment, provided herein is a method which comprises contacting one or more cells in the subject or from the subject with a polypeptide encoded by a nucleic acid having a first nucleotide sequence (e.g., the first nucleotide sequence is identical to or substantially identical to the nucleotide sequence of SEQ ID NO: 1-12). Genomic DNA in the subject comprises a second nucleotide sequence having one or more polymorphic variations associated with osteoarthritis (e.g., the second nucleotide sequence is identical to or substantially identical to a nucleotide sequence of SEQ ID NO: 1-4). The first and second nucleotide sequences typically are substantially identical to one another, and the first nucleotide sequence comprises fewer polymorphic variations associated with osteoarthritis than the second nucleotide sequence. The first nucleotide sequence may comprise a gene sequence that encodes a full-length polypeptide or a fragment thereof. The subject is often a human.

**[0189]** For antibody-based therapies, antibodies can be generated that are both specific for target molecules and that reduce target molecule activity. Such antibodies may be administered in instances where antagonizing a target molecule function is appropriate for the treatment of osteoarthritis.

**[0190]** In circumstances where stimulating antibody production in an animal or a human subject by injection with a target molecule is harmful to the subject, it is possible to generate an immune response against the target molecule by use of anti-idiotypic antibodies (*see, e.g., Herlyn, Ann. Med.; 31(1): 66-78 (1999); and Bhattacharya-Chatterjee & Foon, Cancer Treat. Res.; 94: 51-68 (1998)*). Introducing an anti-idiotypic antibody to a mammal or human subject often stimulates production of anti-anti-idiotypic antibodies, which typically are specific to the target molecule. Vaccines directed to osteoarthritis also may be generated in this fashion.

**[0191]** In instances where the target molecule is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population (*see, e.g., Marasco et al., Proc. Natl. Acad. Sci. USA 90: 7889-7893 (1993)*).

**[0192]** Modulators can be administered to a patient at therapeutically effective doses to treat osteoarthritis. A therapeutically effective dose refers to an amount of the modulator sufficient to result in amelioration of symptoms of osteoarthritis. Toxicity and therapeutic efficacy of modulators can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g., for*

determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Modulators that exhibit large therapeutic indices are preferred. While modulators that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such molecules to the site of affected tissue in order to minimize potential damage to uninfected cells, thereby reducing side effects.

[0193] Data obtained from cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods described herein, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

[0194] Another example of effective dose determination for an individual is the ability to directly assay levels of “free” and “bound” compound in the serum of the test subject. Such assays may utilize antibody mimics and/or “biosensors” that have been created through molecular imprinting techniques. Molecules that modulate target molecule activity are used as a template, or “imprinting molecule”, to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated “negative image” of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell *et al.*, *Current Opinion in Biotechnology* 7: 89-94 (1996) and in Shea, *Trends in Polymer Science* 2: 166-173 (1994). Such “imprinted” affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, *et al.*, *Nature* 361: 645-647 (1993). Through the use of isotope-labeling, the “free” concentration of compound which modulates target molecule expression or activity readily can be monitored and used in calculations of IC<sub>50</sub>. Such “imprinted” affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes readily can be assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC<sub>50</sub>. An example of such a “biosensor” is discussed in Kriz *et al.*, *Analytical Chemistry* 67: 2142-2144 (1995).

[0195] The examples set forth below are intended to illustrate but not limit the invention.

### Examples

[0196] In the following studies a group of subjects was selected according to specific parameters relating to osteoarthritis. Nucleic acid samples obtained from individuals in the study group were subjected to genetic analysis, which identified associations between osteoarthritis and polymorphisms in the following genes or regions: *chromosome 6 (6q27)*, *ELP3*, *CHDC1*, and *ERG* (herein referred to as “targets”). The polymorphisms were genotyped again in two replication cohorts consisting of individuals selected for OA. In addition, SNPs proximal to the incident polymorphisms were identified and allelotyped in OA case and control pools. Methods are described for producing *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1*, and *ERG* polypeptide and polypeptide variants thereof *in vitro* or *in vivo*; *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1*, and *ERG* nucleic acids or polypeptides and variants thereof are utilized for screening test molecules for those that interact with *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1*, and *ERG* molecules. Test molecules identified as interactors with *PSMB1*, *TBP*, *PDCD2*, *ELP3*, *CHDC1*, and *ERG* molecules and variants thereof are further screened *in vivo* to determine whether they treat osteoarthritis.

#### Example 1

##### Samples and Pooling Strategies

##### Sample Selection

[0197] Blood samples were collected from individuals diagnosed with knee osteoarthritis, which were referred to as case samples. Also, blood samples were collected from individuals not diagnosed with knee osteoarthritis as gender and age-matched controls. A database was created that listed all phenotypic trait information gathered from individuals for each case and control sample. Genomic DNA was extracted from each of the blood samples for genetic analyses.

##### DNA Extraction from Blood Samples

[0198] Six to ten milliliters of whole blood was transferred to a 50 ml tube containing 27 ml of red cell lysis solution (RCL). The tube was inverted until the contents were mixed. Each tube was incubated for 10 minutes at room temperature and inverted once during the incubation. The tubes were then centrifuged for 20 minutes at 3000 x g and the supernatant was carefully poured off. 100-200 µl of residual liquid was left in the tube and was pipetted repeatedly to resuspend the pellet in the residual supernatant. White cell lysis solution (WCL) was added to the tube and pipetted repeatedly until completely mixed. While no incubation was normally required, the solution was incubated at 37°C or

room temperature if cell clumps were visible after mixing until the solution was homogeneous. 2 ml of protein precipitation was added to the cell lysate. The mixtures were vortexed vigorously at high speed for 20 sec to mix the protein precipitation solution uniformly with the cell lysate, and then centrifuged for 10 minutes at 3000 x g. The supernatant containing the DNA was then poured into a clean 15 ml tube, which contained 7 ml of 100% isopropanol. The samples were mixed by inverting the tubes gently until white threads of DNA were visible. Samples were centrifuged for 3 minutes at 2000 x g and the DNA was visible as a small white pellet. The supernatant was decanted and 5 ml of 70% ethanol was added to each tube. Each tube was inverted several times to wash the DNA pellet, and then centrifuged for 1 minute at 2000 x g. The ethanol was decanted and each tube was drained on clean absorbent paper. The DNA was dried in the tube by inversion for 10 minutes, and then 1000 µl of 1X TE was added. The size of each sample was estimated, and less TE buffer was added during the following DNA hydration step if the sample was smaller. The DNA was allowed to rehydrate overnight at room temperature, and DNA samples were stored at 2-8°C.

[0199] DNA was quantified by placing samples on a hematology mixer for at least 1 hour. DNA was serially diluted (typically 1:80, 1:160, 1:320, and 1:640 dilutions) so that it would be within the measurable range of standards. 125 µl of diluted DNA was transferred to a clear U-bottom microtitre plate, and 125 µl of 1X TE buffer was transferred into each well using a multichannel pipette. The DNA and 1X TE were mixed by repeated pipetting at least 15 times, and then the plates were sealed. 50 µl of diluted DNA was added to wells A5-H12 of a black flat bottom microtitre plate. Standards were inverted six times to mix them, and then 50 µl of 1X TE buffer was pipetted into well A1, 1000 ng/ml of standard was pipetted into well A2, 500 ng/ml of standard was pipetted into well A3, and 250 ng/ml of standard was pipetted into well A4. PicoGreen (Molecular Probes, Eugene, Oregon) was thawed and freshly diluted 1:200 according to the number of plates that were being measured. PicoGreen was vortexed and then 50µl was pipetted into all wells of the black plate with the diluted DNA. DNA and PicoGreen were mixed by pipetting repeatedly at least 10 times with the multichannel pipette. The plate was placed into a Fluoroskan Ascent Machine (microplate fluorometer produced by Labsystems) and the samples were allowed to incubate for 3 minutes before the machine was run using filter pairs 485 nm excitation and 538 nm emission wavelengths. Samples having measured DNA concentrations of greater than 450 ng/µl were re-measured for conformation. Samples having measured DNA concentrations of 20 ng/µl or less were re-measured for confirmation.

#### Pooling Strategies – Discovery Cohort

[0200] Samples were derived from the Nottingham knee OA family study (UK) where index cases were identified through a knee replacement registry. Siblings were approached and assessed with knee x-

rays and assigned status as affected or unaffected. In all 1,157 individuals were available. In order to create same-sex pools of appropriate sizes, 335 unrelated female individuals with OA from the Nottingham OA sample were selected for the case pool. The control pool was made up of unrelated female individuals from the St. Thomas twin study (England) with normal knee x-rays and without other indications of OA, regardless of anatomical location, as well as lacking family history of OA. The St. Thomas twin study consists of Caucasian, female participants from the St. Thomas' Hospital, London, adult-twin registry, which is a voluntary registry of >4,000 twin pairs ranging from 18 to 76 years of age. The female case samples and female control samples are described further in Table 1 below.

**[0201]** A select set of samples from each group were utilized to generate pools, and one pool was created for each group. Each individual sample in a pool was represented by an equal amount of genomic DNA. For example, where 25 ng of genomic DNA was utilized in each PCR reaction and there were 200 individuals in each pool, each individual would provide 125 pg of genomic DNA. Inclusion or exclusion of samples for a pool was based upon the following criteria: the sample was derived from an individual characterized as Caucasian; the sample was derived from an individual of British paternal and maternal descent; case samples were derived from individuals diagnosed with specific knee osteoarthritis (OA) and were recruited from an OA knee replacement clinic. Control samples were derived from individuals free of OA, family history of OA, and rheumatoid arthritis. Also, sufficient genomic DNA was extracted from each blood sample for all allelotyping and genotyping reactions performed during the study. Phenotype information from each individual was collected and included age of the individual, gender, family history of OA, general medical information (e.g., height, weight, thyroid disease, diabetes, psoriasis, hysterectomy), joint history (previous and current symptoms, joint-related operations, age at onset of symptoms, date of primary diagnosis, age of individual as of primary diagnosis and order of involvement), and knee-related findings (crepitus, restricted passive movement, bony swelling/deformity). Additional knee information included knee history, current symptoms, any major knee injury, menisectomy, knee replacement surgery, age of surgery, and treatment history (including hormone replace therapy (HRT)). Samples that met these criteria were added to appropriate pools based on disease status.

**[0202]** The selection process yielded the pools set forth in Table 1, which were used in the studies that follow:

**TABLE 1**

	<b>Female case</b>	<b>Female control</b>
<b>Pool size (Number)</b>	335	335
<b>Pool Criteria (ex: case/control)</b>	control	case

<b>Mean Age</b> (ex: years)	57.21	69.95
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## Example 2

### Association of Polymorphic Variants with Osteoarthritis

[0203] A whole-genome screen was performed to identify particular SNPs associated with occurrence of osteoarthritis. As described in Example 1, two sets of samples were utilized, which included samples from female individuals having knee osteoarthritis (osteoarthritis cases), and samples from female individuals not having knee osteoarthritis (female controls). The initial screen of each pool was performed in an allelotyping study, in which certain samples in each group were pooled. By pooling DNA from each group, an allele frequency for each SNP in each group was calculated. These allele frequencies were then compared to one another. Particular SNPs were considered as being associated with osteoarthritis when allele frequency differences calculated between case and control pools were statistically significant. SNP disease association results obtained from the allelotyping study were then validated by genotyping each associated SNP across all samples from each pool. The results of the genotyping then were analyzed, allele frequencies for each group were calculated from the individual genotyping results, and a p-value was calculated to determine whether the case and control groups had statistically significant differences in allele frequencies for a particular SNP. When the genotyping results agreed with the original allelotyping results, the SNP disease association was considered validated at the genetic level.

### SNP Panel Used for Genetic Analyses

[0204] A whole-genome SNP screen began with an initial screen of approximately 25,000 SNPs over each set of disease and control samples using a pooling approach. The pools studied in the screen are described in Example 1. The SNPs analyzed in this study were part of a set of 25,488 SNPs confirmed as being statistically polymorphic as each is characterized as having a minor allele frequency of greater than 10%. The SNPs in the set reside in genes or in close proximity to genes, and many reside in gene exons. Specifically, SNPs in the set are located in exons, introns, and within 5,000 base-pairs upstream of a transcription start site of a gene. In addition, SNPs were selected according to the following criteria: they are located in ESTs; they are located in Locuslink or Ensembl genes; and they are located in Genomatix promoter predictions. SNPs in the set were also selected on the basis of even spacing across the genome, as depicted in Table 2.

[0205] A case-control study design using a whole genome association strategy involving approximately 28,000 single nucleotide polymorphisms (SNPs) was employed. Approximately 25,000

SNPs were evenly spaced in gene-based regions of the human genome with a median inter-marker distance of about 40,000 base pairs. Additionally, approximately 3,000 SNPs causing amino acid substitutions in genes described in the literature as candidates for various diseases were used. The case-control study samples were of female Caucasian origin (British paternal and maternal descent) 670 individuals were equally distributed in two groups: female controls and female cases. The whole genome association approach was first conducted on 2 DNA pools representing the 2 groups. Significant markers were confirmed by individual genotyping.

**TABLE 2**

<u>General Statistics</u>		<u>Spacing Statistics</u>	
Total # of SNPs	25,488	Median	37,058 bp
# of Exonic SNPs	>4,335 (17%)	Minimum*	1,000 bp
# SNPs with refSNP ID	20,776 (81%)	Maximum*	3,000,000 bp
Gene Coverage	>10,000	Mean	122,412 bp
Chromosome Coverage	All	Std Deviation	373,325 bp
		<i>*Excludes outliers</i>	

#### Allelotyping and Genotyping Results

[0206] The genetic studies summarized above and described in more detail below identified allelic variants in the target genes that are associated with osteoarthritis.

#### Assay for Verifying, Allelotyping, and Genotyping SNPs

[0207] A MassARRAY™ system (Sequenom, Inc.) was utilized to perform SNP genotyping in a high-throughput fashion. This genotyping platform was complemented by a homogeneous, single-tube assay method (hME™ or homogeneous MassEXTEND™ (Sequenom, Inc.)) in which two genotyping primers anneal to and amplify a genomic target surrounding a polymorphic site of interest. A third primer (the MassEXTEND™ primer), which is complementary to the amplified target up to but not including the polymorphism, was then enzymatically extended one or a few bases through the polymorphic site and then terminated.

[0208] For each polymorphism, SpectroDESIGNER™ software (Sequenom, Inc.) was used to generate a set of PCR primers and a MassEXTEND™ primer which were used to genotype the polymorphism. Other primer design software could be used or one of ordinary skill in the art could manually design primers based on his or her knowledge of the relevant factors and considerations in designing such primers. Table 3 shows PCR primers and Table 4 shows extension primers used for analyzing polymorphisms. The initial PCR amplification reaction was performed in a 5 µl total volume containing 1X PCR buffer with 1.5 mM MgCl<sub>2</sub> (Qiagen), 200 µM each of dATP, dGTP, dCTP, dTTP

(Gibco-BRL), 2.5 ng of genomic DNA, 0.1 units of HotStar DNA polymerase (Qiagen), and 200 nM each of forward and reverse PCR primers specific for the polymorphic region of interest.

**TABLE 3: PCR Primers**

SNP Reference	Forward PCR primer	Reverse PCR primer
rs756519	ACGTTGGATGTCTAGAGACACCTGAGGTTG	ACGTTGGATGTGTTTCACTTCAGAGCCCTG
rs1042327	ACGTTGGATGAACTTCACATCACAGCTCCC	ACGTTGGATGCAGAAGTTGGGTTTTCCAGC
rs8770	ACGTTGGATGCTGTCACTGGACACTTTTG	ACGTTGGATGAAAATAGAGGTGCAGAGATG
rs1563055	ACGTTGGATGAGTTCTTTCTCCTCACATTG	ACGTTGGATGCCCTTTAGAAGCACATACTC
rs912428	ACGTTGGATGACTACATCCATTCCAGGGAG	ACGTTGGATGTCAGATCAGAGTGAGTTTAG
rs1888475	ACGTTGGATGACCCCTGGCAAGTGAATTAC	ACGTTGGATGGGGAGGTGGATGTTCTTATC

[0209] Samples were incubated at 95°C for 15 minutes, followed by 45 cycles of 95°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minute, finishing with a 3 minute final extension at 72°C. Following amplification, shrimp alkaline phosphatase (SAP) (0.3 units in a 2 µl volume) (Amersham Pharmacia) was added to each reaction (total reaction volume was 7 µl) to remove any residual dNTPs that were not consumed in the PCR step. Samples were incubated for 20 minutes at 37°C, followed by 5 minutes at 85°C to denature the SAP.

[0210] Once the SAP reaction was complete, a primer extension reaction was initiated by adding a polymorphism-specific MassEXTEND™ primer cocktail to each sample. Each MassEXTEND™ cocktail included a specific combination of dideoxynucleotides (ddNTPs) and deoxynucleotides (dNTPs) used to distinguish polymorphic alleles from one another. Methods for verifying, allelotyping and genotyping SNPs are disclosed, for example, in U.S. Pat. No. 6,258,538, the content of which is hereby incorporated by reference. In Table 4, ddNTPs are shown and the fourth nucleotide not shown is the dNTP.

**TABLE 4: Extension Primers**

SNP Reference	Extend Probe	Termination Mix
rs756519	CAGAGCCCTGTTCTTTGATT	ACG
rs1042327	CATCACAGCTCCCCACCAT	ACT
rs8770	TAGACACTGTGTAAGCAATC	ACG
rs1563055	TTCTCCTCACATTGTTTCTACT	ACG
rs912428	CCATTCCAGGGAGACTCCCA	ACT
rs1888475	GACATCAAATGATTCCCCTGT	ACT

[0211] The MassEXTEND™ reaction was performed in a total volume of 9 µl, with the addition of 1X ThermoSequenase buffer, 0.576 units of ThermoSequenase (Amersham Pharmacia), 600 nM MassEXTEND™ primer, 2 mM of ddATP and/or ddCTP and/or ddGTP and/or ddTTP, and 2 mM of dATP or dCTP or dGTP or dTTP. The deoxy nucleotide (dNTP) used in the assay normally was

complementary to the nucleotide at the polymorphic site in the amplicon. Samples were incubated at 94°C for 2 minutes, followed by 55 cycles of 5 seconds at 94°C, 5 seconds at 52°C, and 5 seconds at 72°C.

[0212] Following incubation, samples were desalted by adding 16 µl of water (total reaction volume was 25 µl), 3 mg of SpectroCLEAN™ sample cleaning beads (Sequenom, Inc.) and allowed to incubate for 3 minutes with rotation. Samples were then robotically dispensed using a piezoelectric dispensing device (SpectroJET™ (Sequenom, Inc.)) onto either 96-spot or 384-spot silicon chips containing a matrix that crystallized each sample (SpectroCHIP™ (Sequenom, Inc.)). Subsequently, MALDI-TOF mass spectrometry (Biflex and Autoflex MALDI-TOF mass spectrometers (Bruker Daltonics) can be used) and SpectroTYPER RT™ software (Sequenom, Inc.) were used to analyze and interpret the SNP genotype for each sample.

#### Genetic Analysis

[0213] Minor allelic frequencies for the polymorphisms set forth in Table A were verified as being 10% or greater using the extension assay described above in a group of samples isolated from 92 individuals originating from the state of Utah in the United States, Venezuela and France (Coriell cell repositories).

[0214] Genotyping results are shown for female pools in Table 5. In Table 5, “AF” refers to allelic frequency; and “F case” and “F control” refer to female case and female control groups, respectively.

**TABLE 5: Genotyping Results**

SNP Reference	AF F case	AF F control	p-value
rs756519	C = 0.581 T = 0.419	C = 0.656 T = 0.344	0.0055
rs1042327	T = 0.472 C = 0.528	T = 0.563 C = 0.437	0.0012
rs8770	C = 0.529 T = 0.471	C = 0.432 T = 0.568	0.0001
rs1563055	C = 0.653 T = 0.347	C = 0.736 T = 0.264	0.0013
rs912428	T = 0.228 C = 0.772	T = 0.170 C = 0.830	0.0076
rs1888475	A = 0.188 G = 0.812	A = 0.135 G = 0.865	0.0087

[0215] All of the single marker alleles set forth in Table A were considered validated, since the genotyping data agreed with the allelotyping data and each SNP significantly associated with osteoarthritis. Particularly significant associations with osteoarthritis are indicated by a calculated p-value of less than 0.05 for genotype results.

### Example 3

#### Association of Polymorphic Variants with Osteoarthritis in Replication Cohorts

[0216] The single marker polymorphisms set forth in Table A were genotyped again in two replication cohorts consisting of individuals selected for OA.

#### Sample Selection and Pooling Strategies – Replication Sample 1

[0217] A second case control sample (replication sample #1) was created by using 100 Caucasian female cases from Chingford, UK, and 148 unrelated female cases from the St. Thomas twin study. Cases were defined as having Kellgren-Lawrence (KL) scores of at least 2 in at least one knee x-ray. In addition, 199 male knee replacement cases from Nottingham were included. (For a cohort description, see the Nottingham description provided in Example 1). The control pool was made up of unrelated female individuals from the St. Thomas twin study (England) with normal knee x-rays and without other indications of OA, regardless of anatomical location, as well as lacking family history of OA. The St. Thomas twin study consists of Caucasian, female participants from the St. Thomas' Hospital, London, adult-twin registry, which is a voluntary registry of >4,000 twin pairs ranging from 18 to 76 years of age. The replication sample 1 cohort was used to replicate the initial results. Table 6 below summarizes the selected phenotype data collected from the case and control individuals.

**TABLE 6**

<b>Phenotype</b>	<b>Female cases (n=248): median (range)/ (n,%)</b>	<b>Male cases (n=199): median (range)/ (n,%)</b>	<b>Female controls (n=313): mean (range)/ (n,%)</b>
Age	59 (39- 73)	66 (45- 73)	55 (50- 72)
Height (cm)	162 (141- 178)	175 (152- 198)	162 (141- 176)
Weight (kg)	68 (51- 123)	86 (62- 127)	64 (40- 111)
Body mass index (kg/m <sup>2</sup> )	26 (18- 44)	29 (21- 41)	24 (18- 46)
Kellgren- Lawrence* left knee	0 (63, 26%), 1 (20, 8%), 2 (105, 43%), 3 (58, 23%), 4 (1, 0%)	NA	NA
Kellgren- Lawrence* right knee	0 (43, 7%), 1 (18, 7%), 2 (127, 52%), 3 (57, 23%), 4 (1, 0%)	NA	NA
KL* >2 both knees	No (145, 59%), Yes (101, 41%)	NA	NA
KL* >2 either knee	No (0, 0%), Yes (248, 100%)	NA	NA

\* 0: normal, 1: doubtful, 2: definite osteophyte (bony protuberance), 3: joint space narrowing (with or without osteophyte), 4: joint deformity

### Sample Selection and Pooling Strategies – Replication Sample 2

[0218] A third case control sample (replication sample #2) was created by using individuals with symptoms of OA from Newfoundland, Canada. These individuals were recruited and examined by rheumatologists. Affected joints were x-rayed and a final diagnosis of definite or probable OA was made according to American College of Rheumatology criteria by a single rheumatologist to avoid any inter-examiner diagnosis variability. Controls were recruited from volunteers without any symptoms from the musculoskeletal system based on a normal joint exam performed by a rheumatologist. Only cases with a diagnosis of definite OA were included in the study. Only individuals of Caucasian origin were included. The cases consisted of 228 individuals with definite knee OA, 106 individuals with definite hip OA, and 74 individuals with hip OA.

**TABLE 7**

<b>Phenotype</b>	<b>Case</b>	<b>Control</b>
Age at Visit	62.7	52.5
Sex (Female/Male)	227/119	174/101
Knee OA Xray: No	35% (120)	80% (16)
Unknown	1% (4)	0% (0)
Yes	64% (221)	20% (4)
Hip OA Xray: No	63% (215)	80% (16)
Unknown	2% (7)	0% (0)
Yes	35% (121)	20% (4)

### Assay for Verifying, Allelotyping, and Genotyping SNPs

[0219] Genotyping of the replication cohorts described in Tables 6 and 7 was performed using the same methods used for the original genotyping, as described herein. A MassARRAY™ system (Sequenom, Inc.) was utilized to perform SNP genotyping in a high-throughput fashion. This genotyping platform was complemented by a homogeneous, single-tube assay method (hME™ or homogeneous MassEXTEND™ (Sequenom, Inc.)) in which two genotyping primers anneal to and amplify a genomic target surrounding a polymorphic site of interest. A third primer (the MassEXTEND™ primer), which is complementary to the amplified target up to but not including the polymorphism, was then enzymatically extended one or a few bases through the polymorphic site and then terminated.

[0220] For each polymorphism, SpectroDESIGNER™ software (Sequenom, Inc.) was used to generate a set of PCR primers and a MassEXTEND™ primer which were used to genotype the polymorphism. Other primer design software could be used or one of ordinary skill in the art could manually design primers based on his or her knowledge of the relevant factors and considerations in

designing such primers. Table 3 shows PCR primers and Table 4 shows extension probes used for analyzing (*e.g.*, genotyping) polymorphisms in the replication cohorts. The initial PCR amplification reaction was performed in a 5  $\mu$ l total volume containing 1X PCR buffer with 1.5 mM MgCl<sub>2</sub> (Qiagen), 200  $\mu$ M each of dATP, dGTP, dCTP, dTTP (Gibco-BRL), 2.5 ng of genomic DNA, 0.1 units of HotStar DNA polymerase (Qiagen), and 200 nM each of forward and reverse PCR primers specific for the polymorphic region of interest.

**[0221]** Samples were incubated at 95°C for 15 minutes, followed by 45 cycles of 95°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minute, finishing with a 3 minute final extension at 72°C. Following amplification, shrimp alkaline phosphatase (SAP) (0.3 units in a 2  $\mu$ l volume) (Amersham Pharmacia) was added to each reaction (total reaction volume was 7  $\mu$ l) to remove any residual dNTPs that were not consumed in the PCR step. Samples were incubated for 20 minutes at 37°C, followed by 5 minutes at 85°C to denature the SAP.

**[0222]** Once the SAP reaction was complete, a primer extension reaction was initiated by adding a polymorphism-specific MassEXTEND™ primer cocktail to each sample. Each MassEXTEND™ cocktail included a specific combination of dideoxynucleotides (ddNTPs) and deoxynucleotides (dNTPs) used to distinguish polymorphic alleles from one another. Methods for verifying, allelotyping and genotyping SNPs are disclosed, for example, in U.S. Pat. No. 6,258,538, the content of which is hereby incorporated by reference. In Table 7, ddNTPs are shown and the fourth nucleotide not shown is the dNTP.

**[0223]** The MassEXTEND™ reaction was performed in a total volume of 9  $\mu$ l, with the addition of 1X ThermoSequenase buffer, 0.576 units of ThermoSequenase (Amersham Pharmacia), 600 nM MassEXTEND™ primer, 2 mM of ddATP and/or ddCTP and/or ddGTP and/or ddTTP, and 2 mM of dATP or dCTP or dGTP or dTTP. The deoxy nucleotide (dNTP) used in the assay normally was complementary to the nucleotide at the polymorphic site in the amplicon. Samples were incubated at 94°C for 2 minutes, followed by 55 cycles of 5 seconds at 94°C, 5 seconds at 52°C, and 5 seconds at 72°C.

**[0224]** Following incubation, samples were desalted by adding 16  $\mu$ l of water (total reaction volume was 25  $\mu$ l), 3 mg of SpectroCLEAN™ sample cleaning beads (Sequenom, Inc.) and allowed to incubate for 3 minutes with rotation. Samples were then robotically dispensed using a piezoelectric dispensing device (SpectroJET™ (Sequenom, Inc.)) onto either 96-spot or 384-spot silicon chips containing a matrix that crystallized each sample (SpectroCHIP™ (Sequenom, Inc.)). Subsequently, MALDI-TOF mass spectrometry (Biflex and Autoflex MALDI-TOF mass spectrometers (Bruker Daltonics) can be used) and

SpectroTYPER RT™ software (Sequenom, Inc.) were used to analyze and interpret the SNP genotype for each sample.

#### Genetic Analysis

[0225] Genotyping results for replication cohorts #1 and #2 are provided in Tables 8 and 9, respectively.

**TABLE 8**

rsID	Replication #1 (Mixed Male/Female cases and Female controls)				Meta-analysis Disc. + Rep #1
	AF OA Con	AF OA Cas	Delta	P-value	P-value
rs756519	0.4	0.43	-0.04	0.140	0.0098
rs1042327	0.49	0.52	-0.03	0.234	0.0430
rs8770	0.51	0.48	0.03	0.303	0.0480
rs1563055	0.31	0.35	-0.04	0.083	0.0002
rs912428	0.86	0.8	0.06	<b>0.004</b>	~0.00001
rs1888475	0.86	0.81	0.04	0.032	0.0002

**TABLE 9**

rsID	Replication #2 (Newfoundland) (Male/Female cases and controls)				Meta-analysis Disc. + Rep #2
	AF OA Con	AF OA Cas	Delta	P-value	Not Done
rs756519	0.39	0.40	-0.007	0.816	
rs1042327	0.49	0.51	-0.024	0.405	
rs8770	0.53	0.49	0.039	0.195	
rs1563055	0.34	0.34	-0.005	0.864	
rs912428	0.82	0.76	0.058	<b>0.016</b>	
rs1888475	0.80	0.82	-0.025	0.280	

[0226] To combine the evidence for association from multiple sample collections, a meta-analysis procedure was employed. The allele frequencies were compared between cases and controls within the discovery sample, as well as within the replication cohort #1 using the DerSimian-Laird approach (DerSimonian, R. and N. Laird. 1986. Meta-analysis in clinical trials. Control Clin Trials 7: 177-188.)

[0227] The absence of a statistically significant association in one or more of the replication cohorts should not be interpreted as minimizing the value of the original finding. There are many reasons why a biologically derived association identified in a sample from one population would not replicate in a sample from another population. The most important reason is differences in population history. Due to bottlenecks and founder effects, there may be common disease predisposing alleles present in one population that are relatively rare in another, leading to a lack of association in the candidate region. Also, because common diseases such as arthritis-related disorders are the result of susceptibilities in

many genes and many environmental risk factors, differences in population-specific genetic and environmental backgrounds could mask the effects of a biologically relevant allele. For these and other reasons, statistically strong results in the original, discovery sample that did not replicate in one or more of the replication samples may be further evaluated in additional replication cohorts and experimental systems.

#### Example 4

##### Chromosome 6 Region Proximal SNPs

**[0228]** It has been discovered that SNPs rs756519, rs1042327 and rs8770 on chromosome 6 (6q27) are associated with occurrence of osteoarthritis in subjects. This region contains genes that encode proteasome (prosome, macropain) subunit, beta type, 1 (*PSMB1*), TATA box binding protein (*TBP*), and programmed cell death 2 (*PDCD2*).

**[0229]** One hundred-nine additional allelic variants proximal to rs756519, rs1042327 and rs8770 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 10. The chromosome positions provided in column four of Table 10 are based on Genome “Build 34” of NCBI’s GenBank.

**TABLE 10**

dbSNP rs#	Chromosome	Position in SEQ ID NO: 1	Chromosome Position	Allele Variants
rs1474555	6	229	170689279	c/t
rs1474554	6	6310	170695360	a/g
rs10334	6	11840	170700890	g/t
rs10541	6	11870	170700920	a/t
rs3823299	6	12064	170701114	a/g
Rs742348	6	13392	170702442	c/g
rs1474644	6	16354	170705404	a/g
rs1474643	6	16559	170705609	c/t
rs2056970	6	16935	170705985	a/g
rs2223474	6	17616	170706666	c/t
rs2206284	6	17737	170706787	c/t
Rs756519	6	18321	170707371	c/t
Rs756518	6	18453	170707503	a/g
Rs756517	6	18811	170707861	c/t
rs1474642	6	20020	170709070	c/t
rs2038093	6	21662	170710712	c/g
rs2038092	6	23197	170712247	c/g
rs2223473	6	23446	170712496	g/t
Rs760909	6	24339	170713389	g/t
rs2076319	6	25504	170714554	a/g
rs3778589	6	27174	170716224	a/g
rs3800236	6	28008	170717058	a/t

dbSNP rs#	Chromosome	Position in SEQ ID NO: 1	Chromosome Position	Allele Variants
rs2206286	6	29294	170718344	c/t
rs12717	6	29759	170718809	c/g
rs2179373	6	30832	170719882	a/g
rs3800235	6	44512	170733562	a/c
rs3823298	6	44850	170733900	c/g
rs2076318	6	45884	170734934	a/g
rs2235506	6	46345	170735395	c/t
rs2072916	6	48589	170737639	a/g
rs3734763	6	53371	170742421	a/g
rs3177571	6	53911	170742961	g/t
rs8770	6	53990	170743040	a/g
rs3173219	6	55152	170744202	c/g
Rs960744	6	55667	170744717	c/t
rs2066954	6	58952	170748002	a/c
rs2072917	6	59315	170748365	g/t
rs3173220	6	60029	170749079	a/g
Rs734249	6	61477	170750527	a/c
rs2092310	6	62988	170752038	c/t
rs2092309	6	63090	170752140	c/g
rs1016536	6	64021	170753071	a/c
rs2235506	6	65685	170754735	c/t
rs2076998	6	70220	170759270	a/g
rs2076997	6	70323	170759373	a/c
rs2345478	6	70959	170760009	a/c
rs2021899	6	73436	170762486	c/g
rs2021898	6	82945	170771995	a/g
rs2345682	6	82958	170772008	g/t
rs2345683	6	82961	170772011	c/g
rs2881195	6	82964	170772014	c/t
rs2345684	6	82965	170772015	g/t
rs3046261	6	83006	170772056	-/cttt
rs4083413	6	83025	170772075	c/t
rs4083412	6	83034	170772084	a/g
rs2345685	6	83074	170772124	g/t
rs2021897	6	83132	170772182	g/t
rs4036211	6	83155	170772205	c/t
rs4036212	6	83172	170772222	a/t
rs4036213	6	83174	170772224	g/t
rs2345686	6	83206	170772256	c/t
rs4036214	6	83216	170772266	g/t
rs4036215	6	83234	170772284	g/t
rs2345687	6	83252	170772302	a/g
rs2345688	6	83260	170772310	a/c
rs2881196	6	83263	170772313	a/c
rs3046288	6	83296	170772346	-/at
rs4036216	6	83319	170772369	a/g
rs4036205	6	83322	170772372	c/g

dbSNP rs#	Chromosome	Position in SEQ ID NO: 1	Chromosome Position	Allele Variants
rs2092307	6	83324	170772374	a/c
rs4036206	6	83357	170772407	c/g
rs2345689	6	83375	170772425	c/t
rs2345690	6	83381	170772431	c/t
rs2345691	6	83389	170772439	a/t
rs2345692	6	83443	170772493	a/g
rs3046306	6	83499	170772549	-/gggtg
rs4036207	6	83545	170772595	c/t
rs2345693	6	83566	170772616	c/t
rs2345694	6	83591	170772641	c/t
rs2345695	6	83619	170772669	g/t
rs2345696	6	83698	170772748	a/g
rs4036209	6	83780	170772830	g/t
rs2345697	6	83784	170772834	g/t
rs2881197	6	83826	170772876	g/t
rs2345698	6	83832	170772882	c/t
rs2345699	6	83852	170772902	c/t
rs2744640	6	86297	170775347	c/t
rs2744639	6	86315	170775365	g/t
rs2744638	6	86420	170775470	c/g
rs2744637	6	86460	170775510	c/g
rs2744636	6	86714	170775764	c/t
rs2744635	6	86718	170775768	c/t
rs2744634	6	86736	170775786	c/g
rs2744633	6	86753	170775803	c/t
rs2744632	6	86766	170775816	g/t
rs2744630	6	88162	170777212	c/g
rs2744629	6	88218	170777268	a/g
rs2744628	6	88246	170777296	a/g
rs2744627	6	88255	170777305	c/t
rs2977616	6	88309	170777359	g/t
rs2977617	6	88310	170777360	a/t
rs2744626	6	88471	170777521	a/g
rs2744625	6	88619	170777669	c/t
rs3115847	6	88904	170777954	c/t
rs2744623	6	89044	170778094	c/g
rs4036193	6	90531	170779581	-/aaaaa
rs4036194	6	90534	170779584	a/g
rs4036196	6	90613	170779663	c/g
Rs1042327	6	46252	170735302	c/t

Assay for Verifying and Allelotyping SNPs

[0230] The methods used to verify and allelotype the 109 proximal SNPs of Table 10 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 11 and Table 12, respectively.

**TABLE 11**

dbSNP rs#	Forward PCR primer	Reverse PCR primer
Rs1474555	ACGTTGGATGACATCAACTGAAGCCGACAG	ACGTTGGATGAATGGTGGAAATGTGATGAGA
Rs1474554	ACGTTGGATGATACACCTAGGACACCTCCA	ACGTTGGATGCAGAAGGAGATAAACCCAGC
rs10334	ACGTTGGATGAACAGTTTCCTCCCTGATGC	ACGTTGGATGCGGCTGGTGAAAGATGTCTT
rs10541	ACGTTGGATGACTATGCAGATCCGGAGTGC	ACGTTGGATGGTCCTTGACAGAGCCATG
Rs3823299	ACGTTGGATGCTCATGTGTACGAGGATTTG	ACGTTGGATGGTCTGGAAGGGTCTTTATTC
rs742348	ACGTTGGATGTGTGGATTTTCCAGTGCTCG	ACGTTGGATGCTGTACTTGAACCTCCCAAGC
Rs1474644	ACGTTGGATGGCAAGACAAGCATAATTGGG	ACGTTGGATGTAAAGGGCATTTTGGCTTCC
Rs1474643	ACGTTGGATGTCTCCCAAATTTAAAGTGGC	ACGTTGGATGGATACCAAAGTCTACTTAC
Rs2056970	ACGTTGGATGTGGGACTACAGGAAGAGAAG	ACGTTGGATGCAAAACACAGACCTTCAGCC
Rs2223474	ACGTTGGATGCCAGGGTAAAGAAAAGATCC	ACGTTGGATGAGAGGCTTACCTCCTAAAAG
Rs2206284	ACGTTGGATGTCACATACTAGGTGGATCCC	ACGTTGGATGAAAGAGGAGAACACAGGATG
rs756519	ACGTTGGATGTCTAGAGACACCTGAGGTTG	ACGTTGGATGTGTTTCACTTCAGAGCCCTG
rs756518	ACGTTGGATGCCAGATTAGACTCTCTAAC	ACGTTGGATGAAATAGCTGAGCTGCCATTG
rs756517	ACGTTGGATGCTCGGTTGTTGACTCCTATC	ACGTTGGATGGCGGATGTTAAGAGTCAGAG
Rs1474642	ACGTTGGATGGGAGGTCATACATTAGCTTC	ACGTTGGATGTACCATCTGACACAATTCTC
Rs2038093	ACGTTGGATGGAGACAGAGTTTCACTCTTG	ACGTTGGATGTAATCACTTGAACCCAGGAG
Rs2038092	ACGTTGGATGTTACCTGAGGTCAGGAGTTT	ACGTTGGATGCCACACCCAGCTGATTTTTG
Rs2223473	ACGTTGGATGCCTTTATGTTATTGCTTTCC	ACGTTGGATGCAGGGAAATTTAAGAATAGC
rs760909	ACGTTGGATGGGAAGAGGCAAGCTTAGTTC	ACGTTGGATGGCAGCATTAAACGAATGCCTG
Rs2076319	ACGTTGGATGGACATTTCAATGCCTTTG	ACGTTGGATGCCAACAGCAACTTAAAACTC
Rs3778589	ACGTTGGATGGCAAGAGAGAGAAAAGTTCC	ACGTTGGATGGTGTCTGTCCCATTTCAC
Rs3800236	ACGTTGGATGAGAGAATGAGGCCCTCATTTT	ACGTTGGATGCTCAGTCATTGTTCTTTTTT
Rs2206286	ACGTTGGATGTTAGACGCTAACCTCTAC	ACGTTGGATGAACATAGCCTCTGCTCTGTG
rs12717	ACGTTGGATGAAAATCGCAGCTGCAAAGGG	ACGTTGGATGAGACAGCAAGTGTCCGATCC
Rs2179373	ACGTTGGATGGAAGTGACCTATGCTCACAC	ACGTTGGATGAATGTCACTTCCGCCAGTTC
Rs3800235	ACGTTGGATGCTATGTGTTGATACCTCCAAG	ACGTTGGATGGCTTCATAAATGAAGTGAAC
Rs3823298	ACGTTGGATGGGTGGTTTCTTGTCTTGATG	ACGTTGGATGTTTTGTCCCAGAGCATCTG
Rs2076318	ACGTTGGATGTCCGCCAAATTATTGTAGCC	ACGTTGGATGCTCAGTAGAAATGCATGGGC
Rs2235506	ACGTTGGATGTAACCATGTCAACTGTTCTC	ACGTTGGATGCCACCAACAATTTAGTAGG
Rs2072916	ACGTTGGATGACGCTGGAGTCACTAAGATG	ACGTTGGATGCAGATTAAGGCACAGGCATG
Rs3734763	ACGTTGGATGGCCTTTTGCTTTTCACTGTC	ACGTTGGATGTAAAGAGGCTGGACCTTCAG
Rs3177571	ACGTTGGATGGTCTGTTGTCAATATAGGTG	ACGTTGGATGACAAAAGTGTCCAGTGACAG
rs8770	ACGTTGGATGAATCCCTGTCACTGGACAC	ACGTTGGATGCCAAAAATAGAGGTGCAGAG
Rs3173219	ACGTTGGATGACATAACCACACTGGAGGTG	ACGTTGGATGCCTAGTTTTTCAAGACACGGTC
rs960744	ACGTTGGATGAAAGGCATGTCACAGTTCCC	ACGTTGGATGGCCCTCTGAGTCAGATAAAC
Rs2066954	ACGTTGGATGGAGGTTCTGGGTATAACTTTC	ACGTTGGATGCTACAAACCAAGTAAGCTGATG
Rs2072917	ACGTTGGATGTGCTAGGCACTCACACTATC	ACGTTGGATGAGGCTTGGAAGTTCTCTCTG
Rs3173220	ACGTTGGATGTATCTGGGTTGACAAAGGCG	ACGTTGGATGACATAAGCAGGCTTGTGCAC
rs734249	ACGTTGGATGAGGTGGACACCAGCAGGGAA	ACGTTGGATGTCACCTCTGCACATGTCTTG

dbSNP rs#	Forward PCR primer	Reverse PCR primer
Rs2092310	ACGTTGGATGTTAGTCAGGTAAAGCGGGAC	ACGTTGGATGTCAGTGGAAGGCTGATCAAG
Rs2092309	ACGTTGGATGATCTAATTGCTTCCCCTCCC	ACGTTGGATGCAGCCTTCCACTGAATACAC
Rs1016536	ACGTTGGATGCCCCAAAAATTGGAGACAGG	ACGTTGGATGGGCTGTCATAATCGTGTGTC
Rs2235506	ACGTTGGATGAAGTGATTCTCCTGCCTCAG	ACGTTGGATGTGGTGAACCCCTGTCTCTAC
Rs2076998	ACGTTGGATGGCTCTGTGATTTTCGATGATG	ACGTTGGATGAGCTACTTCTTGCAGGAGTC
Rs2076997	ACGTTGGATGCAGAGCTTCCAAGTGTTTTC	ACGTTGGATGAAAGGAGTGCTTAAAGGAGC
Rs2345478	ACGTTGGATGCCTTCAACAAGTGCTGACAC	ACGTTGGATGATCCAGGCATTATTGCCAGC
Rs2021899	ACGTTGGATGGTTTTGTGGTGGATGATGGG	ACGTTGGATGAGAGTGCCCATATGGACAG
Rs2021898	ACGTTGGATGCGCAAGAACTCCTTGGATG	ACGTTGGATGCCAATTAAAGCCAAGGTCAC
Rs2345682	ACGTTGGATGATTGCAAGAACTCCTTGG	ACGTTGGATGGGAAGAAATCTTACCAGAAC
Rs2345683	ACGTTGGATGATTGCAAGAACTCCTTGG	ACGTTGGATGGGAAGAAATCTTACCAGAAC
Rs2881195	ACGTTGGATGATTGCAAGAACTCCTTGG	ACGTTGGATGGGAAGAAATCTTACCAGAAC
Rs2345684	ACGTTGGATGATTGCAAGAACTCCTTGG	ACGTTGGATGGGAAGAAATCTTACCAGAAC
Rs3046261	ACGTTGGATGCTCCACTCAGACATCAAAAAG	ACGTTGGATGGTGACCTTGGCTTTAATTGG
Rs4083413	ACGTTGGATGGTGACCTTGGCTTTAATTGG	ACGTTGGATGCTCCACTCAGACATCAAAAAG
Rs4083412	ACGTTGGATGGTGACCTTGGCTTTAATTGG	ACGTTGGATGCTCCACTCAGACATCAAAAAG
Rs2345685	ACGTTGGATGGTCTGGTAAGATTTCTTCC	ACGTTGGATGAGTCTTACAATAGATGACTG
Rs2021897	ACGTTGGATGGCAATTATTTACAGAAGCCC	ACGTTGGATGTCCCACACAGTCATCTATTG
Rs4036211	ACGTTGGATGCCATTACAAGTTGGGCAGTT	ACGTTGGATGCTTTCTGATTCTTTTTTTTTCC
Rs4036212	ACGTTGGATGCTTTCTGATTCTTTTTTTTTCC	ACGTTGGATGCCATTACAAGTTGGGCAGTT
Rs4036213	ACGTTGGATGCCATTACAAGTTGGGCAGTT	ACGTTGGATGCTTTCTGATTCTTTTTTTTTCC
Rs2345686	ACGTTGGATGCCATTACAAGTTGGGCAGTT	ACGTTGGATGCTTTCTGATTCTTTTTTTTTCC
Rs4036214	ACGTTGGATGCCATTACAAGTTGGGCAGTT	ACGTTGGATGCTTTCTGATTCTTTTTTTTTCC
Rs4036215	ACGTTGGATGCTTTCTGATTCTTTTTTTTTCC	ACGTTGGATGCCATTACAAGTTGGGCAGTT
Rs2345687	ACGTTGGATGGGATTGTAAGGTGAGACTTG	ACGTTGGATGTTCTCCCCATTACAAGTTG
Rs2345688	ACGTTGGATGAGGGTCCCATCTAAGAATTC	ACGTTGGATGGGATTGTAAGGTGAGACTTG
Rs2881196	ACGTTGGATGAGGGTCCCATCTAAGAATTC	ACGTTGGATGGGATTGTAAGGTGAGACTTG
Rs3046288	ACGTTGGATGCCAAGTTGTAATGGGGAGGA	ACGTTGGATGCAGTTTTTACAGAGGGTCCC
Rs4036216	ACGTTGGATGCTTGTAAATGGGGAGGAAAAAA	ACGTTGGATGTTCTCATTTTAATCTGTCAG
Rs4036205	ACGTTGGATGCTTGTAAATGGGGAGGAAAAAA	ACGTTGGATGTTCTCATTTTAATCTGTCAG
Rs2092307	ACGTTGGATGCTTGTAAATGGGGAGGAAAAAA	ACGTTGGATGTTCTCATTTTAATCTGTCAG
Rs4036206	ACGTTGGATGGACCCTCTGTAAAACTGAC	ACGTTGGATGCCACTGCACCTCAAATCTTC
Rs2345689	ACGTTGGATGTTCCCTGAGTATCTCCCATG	ACGTTGGATGGGGACCCTCTGTAAAACTG
Rs2345690	ACGTTGGATGTTCCCTGAGTATCTCCCATG	ACGTTGGATGGGGACCCTCTGTAAAACTG
Rs2345691	ACGTTGGATGGCCACCTGTTGGAGATTAC	ACGTTGGATGGGGACCCTCTGTAAAACTG
Rs2345692	ACGTTGGATGTACATGGGAGATACTCAGGG	ACGTTGGATGCCACTGCACCTCAAATCTTC
Rs3046306	ACGTTGGATGGTATAACAAACCTTACCCTTG	ACGTTGGATGTAAAGAAAGAAGATTTGAGG
Rs4036207	ACGTTGGATGTATCAATGGAGAATGCGTGG	ACGTTGGATGGGGAGTTAACCAGCAAAAAGC
Rs2345693	ACGTTGGATGTCGACAACAAGAAGAGAAGG	ACGTTGGATGCACATTAGACAAGGGTAAGG
Rs2345694	ACGTTGGATGCTACCTCTCTCGACAACAAG	ACGTTGGATGCTTAAAGTCCACGCATTCTCC
Rs2345695	ACGTTGGATGCGCATTCTCCATTGATAAGAC	ACGTTGGATGCCATTTAAAGCTACCTCTC
Rs2345696	ACGTTGGATGCCTTACACAAGTGTAACCTC	ACGTTGGATGCCCCAAAAATAATGGTAGG
Rs4036209	ACGTTGGATGGGAACACAGTGATAAGACC	ACGTTGGATGGTTTTCACTTTCGTTAGC
Rs2345697	ACGTTGGATGGTTTTCACTTTCGTTAGC	ACGTTGGATGGCCACCCAAAAATAATGG
Rs2881197	ACGTTGGATGGCTGGAGGAAAAACAAGAAC	ACGTTGGATGCCTACCATTATATTTGGGG
Rs2345698	ACGTTGGATGCTGGAGGAAAAACAAGAACTC	ACGTTGGATGCATTATATTTTGGGGTGGCAT
Rs2345699	ACGTTGGATGGCTGGAGGAAAAACAAGAAC	ACGTTGGATGGGGTGGCATATTTTGGTCTT

dbSNP rs#	Forward PCR primer	Reverse PCR primer
Rs2744640	ACGTTGGATGGCAACAGCACTTAGTATGCC	ACGTTGGATGTGTGAAGCTGCAAATCTGGC
Rs2744639	ACGTTGGATGGCAACAGCACTTAGTATGCC	ACGTTGGATGTGTGAAGCTGCAAATCTGGC
Rs2744638	ACGTTGGATGAACCGTGGCAATACCACGTC	ACGTTGGATGTGGGTTTGGGCTGGATTGG
Rs2744637	ACGTTGGATGTGAGTTGACAGCCTCTGCTGG	ACGTTGGATGCACGTCAGTAAGGCAGAGAC
Rs2744636	ACGTTGGATGTCGAGATGACATTGTCACC	ACGTTGGATGTTCCAGGGGTTACGTGTGTG
Rs2744635	ACGTTGGATGTGAGTCTGACTGTGTCACGG	ACGTTGGATGTCGAGATGACATTGTCACC
Rs2744634	ACGTTGGATGCGTGTTCCAGGGATTATATG	ACGTTGGATGGCACATAACGCTTGGAACTC
Rs2744633	ACGTTGGATGTATGAGTGTGACGGGTGTAG	ACGTTGGATGGCACATAACGCTTGGAACTC
Rs2744632	ACGTTGGATGTAGCTGCCTTCCACATCCAA	ACGTTGGATGTGTGACGGGTGTAGCGTTAG
Rs2744630	ACGTTGGATGGGGTTCAAATGCCTCTGATAG	ACGTTGGATGGGTCTAGGACAAGACCCATT
Rs2744629	ACGTTGGATGAACTTTCCTTAGCCAGTGG	ACGTTGGATGATCAGAGGCATTTGAACCCC
Rs2744628	ACGTTGGATGTTGACCTCAAATCATGTCAC	ACGTTGGATGTATCAGAGGCATTTGAACCCC
Rs2744627	ACGTTGGATGGGGTGGTTTATGTTCCACTG	ACGTTGGATGCCAGAACTAATGCTAGCTTC
Rs2977616	ACGTTGGATGTTCCACTGGCTAAGAGAAAG	ACGTTGGATGCCAGAACTAATGCTAGCTTC
Rs2977617	ACGTTGGATGCCAGAACTAATGCTAGCTTC	ACGTTGGATGTTCCACTGGCTAAGAGAAAG
Rs2744626	ACGTTGGATGACAGTGAAATTGTATTTCCG	ACGTTGGATGGCACAAACTTAAGAATCTCC
Rs2744625	ACGTTGGATGAGCAAAATCCACCTATGTCC	ACGTTGGATGCTGAATTTTGTCTCCAGTAC
Rs3115847	ACGTTGGATGTCGAGGCAGAGGCGTAGTA	ACGTTGGATGATAGGAATGACATGAACCCG
Rs2744623	ACGTTGGATGACGCGAGTCCGTAGGTGCTG	ACGTTGGATGAAGAGGCTGCTACCCAGAG
Rs4036193	ACGTTGGATGAGAGCAAGACTCCGTCTCAA	ACGTTGGATGACATGTCGCTTGATGTGTGC
Rs4036194	ACGTTGGATGACATGTCGCTTGATGTGTGC	ACGTTGGATGAGAGCAAGACTCCGTCTCAA
Rs4036196	ACGTTGGATGCCCCAGCGTTTCATATTTGTC	ACGTTGGATGTCTGGCCAAATGGTCATACC
rs1042327	ACGTTGGATGAACTTCACATCACAGCTCCC	ACGTTGGATGCAGAAAGTTGGGTTTCCAGC

**TABLE 12**

dbSNP rs#	Extend Primer	Term Mix
rs1474555	TGAAGCCGACAGTGACACC	ACT
rs1474554	CCAATTTTGCACACCTCCAGCA	ACG
rs10334	CAGATCCGGAGTGCGTCC	CGT
rs10541	TCTCTCTCAGCCGCAGAA	CGT
rs3823299	GAGGATTTGTGATGAAAATACTA	ACG
rs742348	AATCCCCGTGTTGTTCAAGG	ACT
rs1474644	AAGGATGTTTCATCATAGTGTTTA	ACG
rs1474643	ACATGTTTATACATACTCATG	ACG
rs2056970	TTGGCAGCTTTTATAGGCCTC	ACT
rs2223474	AAGTCTCAAAAAGGTCCC	ACT
rs2206284	TAGGTGGATCCCTTTTCCC	ACG
rs756519	CAGAGCCCTGTTCTTTGATTT	ACG
rs756518	CAAAGGATGCTGTCTGGCC	ACG
rs756517	GTTCCATGAGCGTTTCTTTG	ACG
rs1474642	CTTCAGTTTCTTCATCACTTTC	ACT
rs2038093	TTTCACTCTTGTTGCCAGG	ACT
rs2038092	CCAACATGGTGAAACCCCATCT	ACT
rs2223473	TAGAATTAATAATTAGACTTTGGGG	ACT

dbSNP rs#	Extend Primer	Term Mix
rs760909	GCAAGCTTAGTTCTAGGTCAG	CGT
rs2076319	TCACAATGCCTTTGTAATGATTT	ACT
rs3778589	GTTTTAGGAAGACTGCTCTGACAA	ACG
rs3800236	CTGAGAGCCAGCTGCAGTAA	CGT
rs2206286	CCTCGCCGGCTGGCATAA	ACT
rs12717	CCATCCCCAAGTCTCTGCCAG	ACT
rs2179373	TGACCTATGCTCACACTTCTCA	ACG
rs3800235	GTGTTGATACCTCCAAGTACATTT	CGT
rs3823298	CTTGATGAAATAGTCATCCAATA	ACT
rs2076318	TGAATTATCACCATCATCA	ACT
rs2235506	TGTTGCCAATAACAATCA	ACG
rs2072916	TGTGACAAGGGATTCCAC	ACG
rs3734763	CATCTGTAAGCAGGGCCGC	ACG
rs3177571	AAGACTGTGTAGCCTTCCTCTG	ACT
rs8770	GTAGACACTGTGTAAGCAATC	ACG
rs3173219	CACTGGAGGTGGAGAGCA	ACT
rs960744	CCCCATCAGACCTGGCTGT	ACT
rs2066954	TTACAATTTGAGCCTTGAGC	CGT
rs2072917	CTATCCCGACCCGAGAAAC	CGT
rs3173220	GCGATGAAACTGAACTGA	ACT
rs734249	CACCAGCAGGGAAGGTTTG	CGT
rs2092310	TTGAGGTGAGGGCTTCCAG	ACT
rs2092309	TCCCCTCCCCTATTGTTTAC	ACT
rs1016536	AAATTGGAGACAGGTCTCAGT	ACT
rs2235506	CTGGGAGTACAGGTGCGC	ACT
rs2076998	GTTTTTGTATAGTCTGCAGATGC	ACT
rs2076997	ATCCATTTTAATGGGTTGCTAGCT	ACT
rs2345478	ACAACGTACTTATTGGGCATA	ACT
rs2021899	CTTCTTGGAAGCTCTTCCCA	ACT
rs2021898	TTGGATGGGGTTAATGGCAG	ACG
rs2345682	GTTAATGGCAGCTGTATTTTTCTG	ACT
rs2345683	GGCAGCTGTATTTTTCTGTGA	ACT
rs2881195	CAGCTGTATTTTTCTGTGACCT	ACG
rs2345684	GCAGCTGTATTTTTCTGTGACCTT	ACT
rs3046261	GAAAACATTTGAGATACTGAAGAT	ACT
rs4083413	TTCTTTATCTTCAGTATCTCAA	ACT
rs4083412	TCTTCAGTATCTCAAATGTTTTCA	ACG
rs2345685	CAACTTTTGATGTCTGAGTGGA	ACT
rs2021897	ATTATTTACAGAAGCCCTATTCA	ACT
rs4036211	TTCCAAACAAAAGCTACCATGCA	ACT
rs4036212	AAATAATTGCATGGTAGCTTTTG	CGT
rs4036213	ACAACACTTTTGATGTTATTTCC	CGT
rs2345686	ACAATCCAAAAATCACATTCCTA	ACT
rs4036214	GTCTCACCTTACAATCCAAAAAT	CGT

dbSNP rs#	Extend Primer	Term Mix
rs4036215	AATGTGATTTTTGGATTGTAAGG	ACT
rs2345687	AAGGTGAGACTTGTTTAGCTTT	ACT
rs2345688	TCCTCCCCATTACAAGTTGGGCA	ACT
rs2881196	TTTTCCTCCCCATTACAAGTTGG	ACT
rs3046288	TAATGGGGAGGAAAAAAATTTTCT	ACT
rs4036216	ATGTTTTTGAATTCTTAGATGG	ACT
rs4036205	GTTTTTGAATTCTTAGATGGGAC	ACT
rs2092307	TGGAATTCTTAGATGGGACCC	ACT
rs4036206	ACTGACAGATTAAAATGAGAAAAA	ACT
rs2345689	TCCCATGTATCCATAAGGTATAC	ACT
rs2345690	GTATCTCCCATGTATCCATAAG	ACT
rs2345691	CCCTGAGTATCTCCCATGTA	CGT
rs2345692	TCTCCAACAGGTGGCTTTCA	ACT
rs3046306	TTGCTGGTTAACTCCCCACT	CGT
rs4036207	GCGTGGACTTAAGTCTGTATAAC	ACT
rs2345693	AGAGTCTTATCAATGGAGAATGC	ACT
rs2345694	GAAGAGAAGGATAACTAAATCACT	ACT
rs2345695	ATTTAGTTATCCTTCTCTTCTTG	ACT
rs2345696	ACACAAGTGTAACCTCTACTCT	ACT
rs4036209	GGAAACCAGAATATGCCACC	CGT
rs2345697	AGCCAAAGGGACATATTTTGTGGT	ACT
rs2881197	GGAACACAGTGTATAAGACCAAA	CGT
rs2345698	CGGTGGAACACAGTGTATAAG	ACT
rs2345699	AAAACAAGAACTCTTTTCATTGCC	ACT
rs2744640	TTTATCTCCAGTTCCCCAGC	ACG
rs2744639	AGCACTTAGTATGCCTTCTCCTT	ACT
rs2744638	TGGCAATACCACGTCAGTAAG	ACT
rs2744637	GCTGGGCTGGGTTTGGGCTG	ACT
rs2744636	ACCCGTCACACTCATATAATCCC	ACG
rs2744635	ACACATGCGTGTTCCAGGG	ACT
rs2744634	GGGATTATATGAGTGTGACGG	ACT
rs2744633	GGGTGTAGCGTTAGGTGAC	ACT
rs2744632	GCGCACATAACGCTTGGAAC	ACT
rs2744630	CGTGTTAAACTCATGGCCAAAC	ACT
rs2744629	ATAAACCACCCTGGAGTTCAT	ACT
rs2744628	TTGAAGAAAACTTTCCCTTAGCCA	ACT
rs2744627	GTTTATGTTCCACTGGCTAAG	ACT
rs2977616	TTGAGGTCAAACATTAATATCAAG	ACT
rs2977617	CTAGCTTCTCAATCTTTTGAGTT	CGT
rs2744626	GTGAAATTGTATTTCCGGATTTC	ACT
rs2744625	TCCTGAACACTTATCCACTTTAC	ACT
rs3115847	CCAGGGCTGGAGGGGCC	ACT
rs2744623	GGTGCTGGCGGGAGCGAGAGT	ACT
rs4036193	GACTCCGTCTCAAAAAAAAAAAAAA	ACT

dbSNP rs#	Extend Primer	Term Mix
rs4036194	CTTGATGTGTGCTTCAGGGTA	ACG
rs4036196	CAGTGCAAGTAAAGAGCCTTA	ACT
rs1042327	CATCACAGCTCCCCACCAT	ACT

### Genetic Analysis

[0231] Allelotyping results from the discovery cohort are shown for cases and controls in Table 13. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 (A1 AF = 1-A2 AF). For example, the SNP rs1474555 has the following case and control allele frequencies: case A1 (C) = 0.64; case A2 (T) = 0.36; control A1 (C) = 0.70; and control A2 (T) = 0.30, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

**TABLE 13**

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
Rs1474555	229	170689279	C/T	0.36	0.30	<b>0.024</b>
Rs1474554	6310	170695360	A/G	0.48	0.43	0.058
rs10334	11840	170700890	G/T			
rs10541	11870	170700920	A/T			
Rs3823299	12064	170701114	A/G	0.45	0.41	0.125
Rs742348	13392	170702442	C/G	0.46	0.44	0.275
Rs1474644	16354	170705404	A/G	0.75	0.77	0.270
Rs1474643	16559	170705609	C/T	0.45	0.40	<b>0.042</b>
Rs2056970	16935	170705985	A/G	0.36	0.33	0.242
Rs2223474	17616	170706666	C/T	0.42	0.46	0.140
Rs2206284	17737	170706787	C/T	0.37	0.35	0.493
rs756519	18321	170707371	C/T			
Rs756518	18453	170707503	A/G	0.49	0.53	0.133
Rs756517	18811	170707861	C/T			
Rs1474642	20020	170709070	C/T	0.12	0.12	0.904
Rs2038093	21662	170710712	C/G			
Rs2038092	23197	170712247	C/G			
Rs2223473	23446	170712496	G/T	0.42	0.45	0.296
Rs760909	24339	170713389	G/T	0.49	0.52	0.255
Rs2076319	25504	170714554	A/G	0.43	0.46	0.219
Rs3778589	27174	170716224	A/G	0.49	0.54	0.081
Rs3800236	28008	170717058	A/T	0.47	0.50	0.319
Rs2206286	29294	170718344	C/T	0.81	0.82	0.831
rs12717	29759	170718809	C/G	0.52	0.57	0.081
rs2179373	30832	170719882	A/G	0.58	0.62	0.089
rs3800235	44512	170733562	A/C	0.60	0.64	0.077
rs3823298	44850	170733900	C/G	0.44	0.38	<b>0.022</b>
rs2076318	45884	170734934	A/G	0.41	0.45	0.109
rs2235506	46345	170735395	C/T	0.68	0.66	0.320
rs2072916	48589	170737639	A/G	0.48	0.51	0.192
rs3734763	53371	170742421	A/G	0.50	0.54	0.142

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3177571	53911	170742961	G/T			
rs8770	53990	170743040	A/G			
rs3173219	55152	170744202	C/G	0.49	0.53	0.056
rs960744	55667	170744717	C/T	0.39	0.35	0.179
rs2066954	58952	170748002	A/C	0.37	0.32	0.057
rs2072917	59315	170748365	G/T	0.46	0.42	0.153
rs3173220	60029	170749079	A/G			
rs734249	61477	170750527	A/C	0.48	0.40	0.022
rs2092310	62988	170752038	C/T			
rs2092309	63090	170752140	C/G	0.43	0.47	0.165
rs1016536	64021	170753071	A/C	0.10	0.10	0.985
rs2235506	65685	170754735	C/T			
rs2076998	70220	170759270	A/G			
rs2076997	70323	170759373	A/C	0.90	0.90	0.814
rs2345478	70959	170760009	A/C	0.09	0.09	0.947
rs2021899	73436	170762486	C/G	0.46	0.43	0.218
rs2021898	82945	170771995	A/G			
rs2345682	82958	170772008	G/T			
rs2345683	82961	170772011	C/G	0.28	0.34	0.019
rs2881195	82964	170772014	C/T			
rs2345684	82965	170772015	G/T			
rs3046261	83006	170772056	-/CTTT			
rs4083413	83025	170772075	C/T			
rs4083412	83034	170772084	A/G			
rs2345685	83074	170772124	G/T	0.71	0.71	0.835
rs2021897	83132	170772182	G/T			
rs4036211	83155	170772205	C/T			
rs4036212	83172	170772222	A/T			
rs4036213	83174	170772224	G/T			
rs2345686	83206	170772256	C/T			
rs4036214	83216	170772266	G/T			
rs4036215	83234	170772284	G/T			
rs2345687	83252	170772302	A/G	0.55	0.50	0.085
rs2345688	83260	170772310	A/C	0.53	0.52	0.958
rs2881196	83263	170772313	A/C			
rs3046288	83296	170772346	-/AT			
rs4036216	83319	170772369	A/G			
rs4036205	83322	170772372	C/G			
rs2092307	83324	170772374	A/C			
rs4036206	83357	170772407	C/G			
rs2345689	83375	170772425	C/T			
rs2345690	83381	170772431	C/T			
rs2345691	83389	170772439	A/T			
rs2345692	83443	170772493	A/G			
rs3046306	83499	170772549	-/GGTG	0.42	0.43	0.761
rs4036207	83545	170772595	C/T			
rs2345693	83566	170772616	C/T			
rs2345694	83591	170772641	C/T			
rs2345695	83619	170772669	G/T			
rs2345696	83698	170772748	A/G			
rs4036209	83780	170772830	G/T	0.79	0.73	0.156
rs2345697	83784	170772834	G/T			
rs2881197	83826	170772876	G/T			
rs2345698	83832	170772882	C/T			
rs2345699	83852	170772902	C/T			
rs2744640	86297	170775347	C/T	0.53	0.53	0.973
rs2744639	86315	170775365	G/T	0.40	0.40	0.789

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2744638	86420	170775470	C/G	0.39	0.39	0.941
rs2744637	86460	170775510	C/G	0.40	0.42	0.497
rs2744636	86714	170775764	C/T	0.76	0.73	0.271
rs2744635	86718	170775768	C/T	0.03	0.02	0.425
rs2744634	86736	170775786	C/G	0.96	0.94	0.436
rs2744633	86753	170775803	C/T	0.14	0.16	0.409
rs2744632	86766	170775816	G/T	0.80	0.83	0.217
rs2744630	88162	170777212	C/G			
rs2744629	88218	170777268	A/G	0.80	0.80	0.978
rs2744628	88246	170777296	A/G	0.71	0.67	0.206
rs2744627	88255	170777305	C/T	0.32	0.30	0.335
rs2977616	88309	170777359	G/T			
rs2977617	88310	170777360	A/T			
rs2744626	88471	170777521	A/G			
rs2744625	88619	170777669	C/T			
rs3115847	88904	170777954	C/T			
rs2744623	89044	170778094	C/G			
rs4036193	90531	170779581	-/AAAAA			
rs4036194	90534	170779584	A/G			
rs4036196	90613	170779663	C/G			
rs1042327	46252	170735302	C/T	0.45	0.39	0.028

[0232] The chromosome 6 proximal SNPs were also allelotyped in the replication cohorts using the methods described herein and the primers provided in Tables 11 and 12. The replication allelotyping results for replication cohort #1 and replication cohort #2 are provided in Tables 14 and 15, respectively.

TABLE 14

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1474555	229	170689279	C/T	0.37	0.27	0.004
rs1474554	6310	170695360	A/G	0.50	0.42	0.020
rs10334	11840	170700890	G/T			
rs10541	11870	170700920	A/T			
rs3823299	12064	170701114	A/G	0.45	0.40	0.080
rs742348	13392	170702442	C/G	0.47	0.41	0.075
rs1474644	16354	170705404	A/G	0.75	0.79	0.231
rs1474643	16559	170705609	C/T	0.46	0.39	0.028
rs2056970	16935	170705985	A/G	0.38	0.33	0.129
rs2223474	17616	170706666	C/T	0.41	0.48	0.052
rs2206284	17737	170706787	C/T	0.37	0.34	0.342
rs756519	18321	170707371	C/T			
rs756518	18453	170707503	A/G	0.48	0.56	0.013
rs756517	18811	170707861	C/T			
rs1474642	20020	170709070	C/T	0.10	0.13	0.277
rs2038093	21662	170710712	C/G			
rs2038092	23197	170712247	C/G			
rs2223473	23446	170712496	G/T	0.42	0.48	0.070
rs760909	24339	170713389	G/T	0.47	0.54	0.077
rs2076319	25504	170714554	A/G	0.41	0.49	0.017
rs3778589	27174	170716224	A/G	0.50	0.57	0.035
rs3800236	28008	170717058	A/T	0.47	0.52	0.126
rs2206286	29294	170718344	C/T	0.80	0.80	0.952

dbSNP rs#	Position in SEQ ID NO: I	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs12717	29759	170718809	C/G	0.53	0.59	0.059
rs2179373	30832	170719882	A/G	0.57	0.64	0.025
rs3800235	44512	170733562	A/C	0.59	0.65	0.065
rs3823298	44850	170733900	C/G	0.46	0.36	0.003
rs2076318	45884	170734934	A/G	0.40	0.47	0.017
rs2235506	46345	170735395	C/T	0.68	0.65	0.434
rs2072916	48589	170737639	A/G	0.47	0.54	0.026
rs3734763	53371	170742421	A/G	0.49	0.56	0.052
rs3177571	53911	170742961	G/T			
rs8770	53990	170743040	A/G			
rs3173219	55152	170744202	C/G	0.49	0.55	0.069
rs960744	55667	170744717	C/T	0.39	0.34	0.131
rs2066954	58952	170748002	A/C	0.36	0.31	0.096
rs2072917	59315	170748365	G/T	0.46	0.41	0.070
rs3173220	60029	170749079	A/G			
rs734249	61477	170750527	A/C	0.37	NA	0.484
rs2092310	62988	170752038	C/T			
rs2092309	63090	170752140	C/G	0.43	0.49	0.102
rs1016536	64021	170753071	A/C	0.08	0.11	0.277
rs2235506	65685	170754735	C/T			
rs2076998	70220	170759270	A/G			
rs2076997	70323	170759373	A/C	0.89	0.91	0.655
rs2345478	70959	170760009	A/C	0.08	0.09	0.660
rs2021899	73436	170762486	C/G	0.48	0.42	0.081
rs2021898	82945	170771995	A/G			
rs2345682	82958	170772008	G/T			
rs2345683	82961	170772011	C/G	0.32	0.39	0.046
rs2881195	82964	170772014	C/T			
rs2345684	82965	170772015	G/T			
rs3046261	83006	170772056	-/CTTT			
rs4083413	83025	170772075	C/T			
rs4083412	83034	170772084	A/G			
rs2345685	83074	170772124	G/T	0.69	0.70	0.772
rs2021897	83132	170772182	G/T			
rs4036211	83155	170772205	C/T			
rs4036212	83172	170772222	A/T			
rs4036213	83174	170772224	G/T			
rs2345686	83206	170772256	C/T			
rs4036214	83216	170772266	G/T			
rs4036215	83234	170772284	G/T			
rs2345687	83252	170772302	A/G	0.62	NA	NA
rs2345688	83260	170772310	A/C	0.46	0.49	0.383
rs2881196	83263	170772313	A/C			
rs3046288	83296	170772346	-/AT			
rs4036216	83319	170772369	A/G			
rs4036205	83322	170772372	C/G			
rs2092307	83324	170772374	A/C			
rs4036206	83357	170772407	C/G			
rs2345689	83375	170772425	C/T			
rs2345690	83381	170772431	C/T			
rs2345691	83389	170772439	A/T			
rs2345692	83443	170772493	A/G			
rs3046306	83499	170772549	-/GGTG	0.39	0.40	0.729
rs4036207	83545	170772595	C/T			
rs2345693	83566	170772616	C/T			
rs2345694	83591	170772641	C/T			
rs2345695	83619	170772669	G/T			

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2345696	83698	170772748	A/G			
rs4036209	83780	170772830	G/T	0.79	0.73	0.156
rs2345697	83784	170772834	G/T			
rs2881197	83826	170772876	G/T			
rs2345698	83832	170772882	C/T			
rs2345699	83852	170772902	C/T			
rs2744640	86297	170775347	C/T	0.49	0.51	0.583
rs2744639	86315	170775365	G/T	0.45	0.43	0.745
rs2744638	86420	170775470	C/G	0.38	0.38	0.852
rs2744637	86460	170775510	C/G	0.35	0.40	0.216
rs2744636	86714	170775764	C/T	0.71	0.73	0.482
rs2744635	86718	170775768	C/T	0.05	0.03	0.195
rs2744634	86736	170775786	C/G	0.93	0.92	0.601
rs2744633	86753	170775803	C/T	0.19	0.20	0.681
rs2744632	86766	170775816	G/T	0.85	0.90	0.070
rs2744630	88162	170777212	C/G			
rs2744629	88218	170777268	A/G	0.78	0.79	0.891
rs2744628	88246	170777296	A/G	0.68	0.67	0.766
rs2744627	88255	170777305	C/T	0.32	0.30	0.636
rs2977616	88309	170777359	G/T			
rs2977617	88310	170777360	A/T			
rs2744626	88471	170777521	A/G			
rs2744625	88619	170777669	C/T			
rs3115847	88904	170777954	C/T			
rs2744623	89044	170778094	C/G			
rs4036193	90531	170779581	-/AAAAA			
rs4036194	90534	170779584	A/G			
rs4036196	90613	170779663	C/G			
rs1042327	46252	170735302	C/T	0.46	0.37	0.004

TABLE 15

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1474555	229	170689279	C/T	0.35	0.36	0.770
rs1474554	6310	170695360	A/G	0.45	0.44	0.873
rs10334	11840	170700890	G/T			
rs10541	11870	170700920	A/T			
rs3823299	12064	170701114	A/G	untyped	0.43	NA
rs742348	13392	170702442	C/G	0.45	0.47	0.600
rs1474644	16354	170705404	A/G	0.74	0.75	0.775
rs1474643	16559	170705609	C/T	0.43	0.41	0.614
rs2056970	16935	170705985	A/G	0.33	0.33	0.978
rs2223474	17616	170706666	C/T	0.44	0.43	0.944
rs2206284	17737	170706787	C/T	0.36	0.37	0.901
rs756519	18321	170707371	C/T			
rs756518	18453	170707503	A/G	0.50	0.47	0.453
rs756517	18811	170707861	C/T			
rs1474642	20020	170709070	C/T	0.15	0.11	0.147
rs2038093	21662	170710712	C/G			
rs2038092	23197	170712247	C/G			
rs2223473	23446	170712496	G/T	0.43	0.40	0.408
rs760909	24339	170713389	G/T	0.51	0.48	0.506
rs2076319	25504	170714554	A/G	0.44	0.40	0.264
rs3778589	27174	170716224	A/G	0.49	0.48	0.910

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3800236	28008	170717058	A/T	0.48	0.46	0.670
rs2206286	29294	170718344	C/T	0.83	0.84	0.685
rs12717	29759	170718809	C/G	0.51	0.53	0.726
rs2179373	30832	170719882	A/G	0.59	0.58	0.880
rs3800235	44512	170733562	A/C	0.60	0.62	0.632
rs3823298	44850	170733900	C/G	0.41	0.41	0.945
rs2076318	45884	170734934	A/G	0.43	0.42	0.636
rs2235506	46345	170735395	C/T	0.69	0.67	0.594
rs2072916	48589	170737639	A/G	0.49	0.46	0.399
rs3734763	53371	170742421	A/G	0.51	0.51	0.888
rs3177571	53911	170742961	G/T			
rs8770	53990	170743040	A/G			
rs3173219	55152	170744202	C/G	0.48	0.51	0.493
rs960744	55667	170744717	C/T	0.38	0.37	0.738
rs2066954	58952	170748002	A/C	0.37	0.34	0.378
rs2072917	59315	170748365	G/T	0.45	0.45	0.982
rs3173220	60029	170749079	A/G			
rs734249	61477	170750527	A/C	0.46	0.02	
rs2092310	62988	170752038	C/T			
rs2092309	63090	170752140	C/G	0.43	0.44	0.891
rs1016536	64021	170753071	A/C	0.13	0.09	0.173
rs2235506	65685	170754735	C/T			
rs2076998	70220	170759270	A/G			
rs2076997	70323	170759373	A/C	0.92	0.89	0.256
rs2345478	70959	170760009	A/C	0.11	0.10	0.545
rs2021899	73436	170762486	C/G	0.44	0.45	0.797
rs2021898	82945	170771995	A/G			
rs2345682	82958	170772008	G/T			
rs2345683	82961	170772011	C/G	0.23	0.26	0.407
rs2881195	82964	170772014	C/T			
rs2345684	82965	170772015	G/T			
rs3046261	83006	170772056	-/CTTT			
rs4083413	83025	170772075	C/T			
rs4083412	83034	170772084	A/G			
rs2345685	83074	170772124	G/T	0.74	0.71	0.533
rs2021897	83132	170772182	G/T			
rs4036211	83155	170772205	C/T			
rs4036212	83172	170772222	A/T			
rs4036213	83174	170772224	G/T			
rs2345686	83206	170772256	C/T			
rs4036214	83216	170772266	G/T			
rs4036215	83234	170772284	G/T			
rs2345687	83252	170772302	A/G	0.47	0.50	0.457
rs2345688	83260	170772310	A/C	0.61	0.58	0.434
rs2881196	83263	170772313	A/C			
rs3046288	83296	170772346	-/AT			
rs4036216	83319	170772369	A/G			
rs4036205	83322	170772372	C/G			
rs2092307	83324	170772374	A/C			
rs4036206	83357	170772407	C/G			
rs2345689	83375	170772425	C/T			
rs2345690	83381	170772431	C/T			
rs2345691	83389	170772439	A/T			
rs2345692	83443	170772493	A/G			
rs3046306	83499	170772549	-/GGTG			
rs4036207	83545	170772595	C/T			
rs2345693	83566	170772616	C/T			

dbSNP rs#	Position in SEQ ID NO: 1	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2345694	83591	170772641	C/T			
rs2345695	83619	170772669	G/T			
rs2345696	83698	170772748	A/G			
rs4036209	83780	170772830	G/T			
rs2345697	83784	170772834	G/T			
rs2881197	83826	170772876	G/T			
rs2345698	83832	170772882	C/T			
rs2345699	83852	170772902	C/T			
rs2744640	86297	170775347	C/T	0.57	0.55	0.595
rs2744639	86315	170775365	G/T	0.35	0.34	0.752
rs2744638	86420	170775470	C/G	0.41	0.40	0.793
rs2744637	86460	170775510	C/G	0.47	0.46	0.836
rs2744636	86714	170775764	C/T	0.83	NA	
rs2744635	86718	170775768	C/T			
rs2744634	86736	170775786	C/G	untyped	0.97	NA
rs2744633	86753	170775803	C/T	0.09	0.10	0.691
rs2744632	86766	170775816	G/T	0.74	0.72	0.529
rs2744630	88162	170777212	C/G			
rs2744629	88218	170777268	A/G	0.81	0.81	0.959
rs2744628	88246	170777296	A/G	0.74	NA	
rs2744627	88255	170777305	C/T	0.33	0.29	0.341
rs2977616	88309	170777359	G/T			
rs2977617	88310	170777360	A/T			
rs2744626	88471	170777521	A/G			
rs2744625	88619	170777669	C/T			
rs3115847	88904	170777954	C/T			
rs2744623	89044	170778094	C/G			
rs4036193	90531	170779581	-/AAAAA			
rs4036194	90534	170779584	A/G			
rs4036196	90613	170779663	C/G			
rs1042327	46252	170735302	C/T	0.42	0.43	0.880

[0233] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1A for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1A can be determined by consulting Table 13. For example, the left-most X on the left graph is at position 170689279. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0234] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial

regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than  $10^{-8}$  were truncated at that value.

[0235] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is placed at the 3' end of each gene to show the direction of transcription.

#### Example 5

##### ELP3 Region Proximal SNPs

[0236] It has been discovered that SNP rs1563055 in elongation protein 3 homolog (*ELP3*) is associated with occurrence of osteoarthritis in subjects.

[0237] Thirty-three additional allelic variants proximal to rs1563055 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 16. The chromosome positions provided in column four of Table 16 are based on Genome "Build 34" of NCBI's GenBank.

**TABLE 16**

dbSNP rs#	Chromosome	Position in SEQ ID NO: 2	Chromosome Position	Allele Variants
rs1000658	8	211	27927511	c/t
rs1984880	8	473	27927773	c/t
rs999112	8	1536	27928836	c/t
rs735880	8	5639	27932939	c/t
rs2045029	8	17186	27944486	a/g
rs2045028	8	17335	27944635	c/t
rs1947384	8	25029	27952329	c/g
rs1947385	8	25111	27952411	c/t
rs1901744	8	28811	27956111	a/g
rs1901745	8	28863	27956163	a/t
rs971882	8	30809	27958109	a/c
rs1377338	8	40985	27968285	a/c
rs2305452	8	45147	27972447	c/t
rs2305451	8	45282	27972582	a/g
rs2123472	8	46168	27973468	g/t
rs2167768	8	46328	27973628	a/g

dbSNP rs#	Chromosome	Position in SEQ ID NO: 2	Chromosome Position	Allele Variants
rs1563055	8	49077	27976377	a/g
rs2290371	8	51925	27979225	c/t
rs2290370	8	52141	27979441	a/g
rs2290369	8	52168	27979468	c/t
rs2874904	8	60852	27988152	c/t
rs3213997	8	62468	27989768	a/g
rs3213998	8	65572	27992872	g/t
rs1530929	8	79089	28006389	a/c
rs1000275	8	79541	28006841	c/t
rs1000274	8	79790	28007090	c/t
rs3757896	8	90843	28018143	a/g
rs3757895	8	90978	28018278	c/t
rs3757894	8	91052	28018352	c/g
rs3757893	8	91131	28018431	a/g
rs3757892	8	91132	28018432	c/t
rs3757891	8	94439	28021739	a/g
rs3757890	8	94621	28021921	a/t

#### Assay for Verifying and Allelotyping SNPs

[0238] The methods used to verify and allelotype the 33 proximal SNPs of Table 16 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 17 and Table 18, respectively.

TABLE 17

dbSNP rs#	Forward PCR primer	Reverse PCR primer
Rs1000658	ACGTTGGATGTTCTCAAAAAAGAAACACAT	ACGTTGGATGGGGTTATCAGTTTGAGATTC
Rs1984880	ACGTTGGATGCCATTTGCCAATTCCTGTGG	ACGTTGGATGATGGGCTGAAATGTATCCCC
rs999112	ACGTTGGATGCTAAGCACATGCCTTTCTTG	ACGTTGGATGCTATTTTCTACTGGGAGATG
rs735880	ACGTTGGATGTGCCCTTCATTCTCCAACCAC	ACGTTGGATGAACAGAGTGAGACCCATCTG
Rs2045029	ACGTTGGATGAGTCATTGCTAGCTTTCTGG	ACGTTGGATGGGGACTTTAGGGAAGTTATAG
Rs2045028	ACGTTGGATGAGCTTGAGTGAGCCGAGAT	ACGTTGGATGTGAGACAGAGTCTTGCTCTG
Rs1947384	ACGTTGGATGATTCTCCACCGAGAAACCAG	ACGTTGGATGTTGTGGCAGCAAGAAGGAAC
Rs1947385	ACGTTGGATGAAATTTCAACAGTCAACAAT	ACGTTGGATGGTCAGTTTTGAAAAGTATC
Rs1901744	ACGTTGGATGCCTTGATTGAAGAGTAAAGC	ACGTTGGATGATCAAATATTCCTCATCCCC
Rs1901745	ACGTTGGATGCTTCTGCCTTTACCTGTGTC	ACGTTGGATGAAATGAAGCAGCACTCACAG
rs971882	ACGTTGGATGAAGCCCTAATCATTGGTACG	ACGTTGGATGGATGGGTGCTAAAAAGACAC
Rs1377338	ACGTTGGATGCCACATATCTACACATCAAG	ACGTTGGATGAGGGAGATAGGTGGTTAAAG
Rs2305452	ACGTTGGATGCCGTGTTGCAACTAACAGGG	ACGTTGGATGAGACGTTCCCATCCTCCATC
Rs2305451	ACGTTGGATGGCAGAGCCACCAGAGATAAA	ACGTTGGATGTTTTACGACAGGCGGGATTG
Rs2123472	ACGTTGGATGCACTTAGAATTGTTGCTTGG	ACGTTGGATGGCTGTATCTGTGACCTCAAA
Rs2167768	ACGTTGGATGGAATCAACATGACTTGGTGAC	ACGTTGGATGATCTCACTCTAAGTTGCTCC
rs1563055	ACGTTGGATGAGTTCTTTCTCCTCACATTG	ACGTTGGATGCCCTTTAGAAGCACATACTC
Rs2290371	ACGTTGGATGATCCTCTTGGTAGCTTGTC	ACGTTGGATGCTGTCTTGGTTTTACCCCTG

dbSNP rs#	Forward PCR primer	Reverse PCR primer
Rs2290370	ACGTTGGATGCAACCTCTACCTCACTACAC	ACGTTGGATGATGAGGTATCGACACACTGG
Rs2290369	ACGTTGGATGACACACTGGGTATCTGTTCT	ACGTTGGATGTCAGAATCCCCAACCTCTAC
Rs2874904	ACGTTGGATGAAATTCCAGGCTGGGTACAG	ACGTTGGATGTGCTGACCTTAAGTGATCCG
Rs3213997	ACGTTGGATGGGTTGGCTAGAAGAGAAAAA	ACGTTGGATGTACAGTCCTTTTGAAACTAC
Rs3213998	ACGTTGGATGACAGTTTGTGACATAGTAG	ACGTTGGATGAGGCTGAAAAGACATTTCATG
Rs1530929	ACGTTGGATGGGCTTTCCTATATTTCTC	ACGTTGGATGGAATACAGTAAGCCTATGGG
Rs1000275	ACGTTGGATGAACCCAGAAAGCAAAAAGC	ACGTTGGATGCACGCTTGCTAACTTAATGG
Rs1000274	ACGTTGGATGGCCTAAGACAGGATCCAAAC	ACGTTGGATGTTACTGCGTGCCTTAGTACC
Rs3757896	ACGTTGGATGCCTTCAAGCAAGTCAGTTAC	ACGTTGGATGCAGAACTGTGTGACTGATC
Rs3757895	ACGTTGGATGAAAATCATTGGCCAACTGC	ACGTTGGATGCTCCTTAGTATTCTTAGGTG
Rs3757894	ACGTTGGATGAGAAGGGTTGAACAACAAGG	ACGTTGGATGCACCTAAGAATACTAAGGAG
Rs3757893	ACGTTGGATGCCCTTGTGTTCAACCCTTC	ACGTTGGATGCTGCATGTGGATACCTACAC
Rs3757892	ACGTTGGATGTCCTGCATGTGGATACCTAC	ACGTTGGATGCCCTTGTGTTCAACCCTTC
Rs3757891	ACGTTGGATGATGGGCCAATTCTCCATAGG	ACGTTGGATGAGGCCTGTTAAGGAAACCTG
Rs3757890	ACGTTGGATGCAGGTGGATGTAGGCTTAAG	ACGTTGGATGGCACCCTGCCTCTTGTTTT

**TABLE 18**

dbSNP rs#	Extend Primer	Term Mix
rs1000658	AATTGACAATGTTGGGACTGTT	ACG
rs1984880	TGTGGTGTAATAGGAGTTAGTGG	ACT
rs999112	GCACATGCCTTTCTTGGAAGT	ACG
rs735880	AACCTTTACTTGTACTACATGC	ACG
rs2045029	GCTAGCTTTCTGGTAATGAAAAT	ACT
rs2045028	GATCGCACCACTGCACTCCAG	ACG
rs1947384	ATAGCGGCAGTCCAAAAAGC	ACT
rs1947385	TTCAACAGTCAACAATGAAACC	ACT
rs1901744	ATAGTCAAGTATGCAAATGAAGC	ACT
rs1901745	CCTTTACCTGTGTCTTCCCT	CGT
rs971882	CCTAATCATTGGTACGGTCTCA	ACT
rs1377338	AGTATTAGCTCAAATATCACATTG	ACT
rs2305452	CAGGGTAGCAGGCGGCC	ACG
rs2305451	CCACAACTCAGACCACGG	ACT
rs2123472	CAGTTAATGTCAAGAAGCATAG	ACT
rs2167768	ACATGACTTGGTGACAGAAGAA	ACT
rs1563055	TTCTCCTCACATTGTTTCTACT	ACG
rs2290371	GGTAGCTTGTCTTAAATAACCGT	ACT
rs2290370	GGAGCAGGGACTTCTGCCA	ACT
rs2290369	AGTCCCTGCTCCATGTGAC	ACT
rs2874904	GGCTAACGCCTGTAATCCCA	ACT
rs3213997	AGAAAAATATTGTTATGCCACA	ACG
rs3213998	TAGTATTCTCAAATAGAGAGATTC	ACT
rs1530929	TTTCCTCTTTCCAGAATTGTATT	ACT
rs1000275	ATGAGAATATCCTAGAATGAGGCA	ACG

dbSNP rs#	Extend Primer	Term Mix
rs1000274	GAATCATCAGGTCCTGTGCC	ACG
rs3757896	TAATTCTCCTTAAGTAGTTAATTC	ACT
rs3757895	TTGGCCAAACTGCAGGATCT	ACT
rs3757894	AAGGGCCACACAAGCAATTTCAA	ACT
rs3757893	CCAAAGGACATTAGGTGGTG	ACG
rs3757892	TGTGGATACCTACACTGCTC	ACG
rs3757891	AGGATAAGTGTAACGGGGTC	ACT
rs3757890	AGTGACACTCTTACTTCACAC	CGT

### Genetic Analysis

[0239] Allelotyping results from the discovery cohort are shown for cases and controls in Table 19. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 ( $A1\ AF = 1 - A2\ AF$ ). For example, the SNP rs1000658 has the following case and control allele frequencies: case A1 (C) = 0.36; case A2 (T) = 0.64; control A1 (C) = 0.37; and control A2 (T) = 0.63, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

**TABLE 19**

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
Rs1000658	211	27927511	C/T	0.79	0.80	0.591
Rs1984880	473	27927773	C/T	0.47	0.48	0.735
Rs999112	1536	27928836	C/T	0.72	0.72	0.775
Rs735880	5639	27932939	C/T	0.20	0.19	0.561
Rs2045029	17186	27944486	A/G	0.54	0.56	0.361
Rs2045028	17335	27944635	C/T			
Rs1947384	25029	27952329	C/G	0.63	0.60	0.122
Rs1947385	25111	27952411	C/T			
Rs1901744	28811	27956111	A/G	0.18	0.18	0.796
Rs1901745	28863	27956163	A/T	0.14	0.18	0.117
Rs971882	30809	27958109	A/C			
Rs1377338	40985	27968285	A/C	0.28	0.24	0.085
Rs2305452	45147	27972447	C/T	0.31	0.27	0.078
Rs2305451	45282	27972582	A/G	0.48	0.52	0.130
Rs2123472	46168	27973468	G/T	0.42	0.45	0.239
Rs2167768	46328	27973628	A/G	0.38	0.35	0.350
Rs1563055	49077	27976377	A/G			
Rs2290371	51925	27979225	C/T	0.28	0.24	0.039
Rs2290370	52141	27979441	A/G	0.85	0.84	0.551
Rs2290369	52168	27979468	C/T	0.43	0.47	0.138
Rs2874904	60852	27988152	C/T	0.26	0.23	0.132
Rs3213997	62468	27989768	A/G	0.44	0.47	0.201
Rs3213998	65572	27992872	G/T	0.83	0.80	0.223
Rs1530929	79089	28006389	A/C	0.47	0.49	0.556

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
Rs1000275	79541	28006841	C/T	0.86	0.87	0.771
Rs1000274	79790	28007090	C/T	0.54	0.56	0.510
Rs3757896	90843	28018143	A/G			
Rs3757895	90978	28018278	C/T	0.46	0.47	0.874
Rs3757894	91052	28018352	C/G	0.08	0.09	0.709
Rs3757893	91131	28018431	A/G	0.16	0.15	0.590
Rs3757892	91132	28018432	C/T	0.09	0.08	0.595
Rs3757891	94439	28021739	A/G			
Rs3757890	94621	28021921	A/T	0.98	0.96	0.167

[0240] The *ELP3* proximal SNPs were also allelotyped in the replication cohorts using the methods described herein and the primers provided in Tables 17 and 18. The replication allelotyping results for replication cohort #1 and replication cohort #2 are provided in Tables 20 and 21, respectively.

**TABLE 20**

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1000658	211	27927511	C/T	0.78	0.79	0.863
rs1984880	473	27927773	C/T	0.46	0.48	0.594
rs999112	1536	27928836	C/T	0.71	0.70	0.759
rs735880	5639	27932939	C/T	0.20	0.17	0.255
rs2045029	17186	27944486	A/G	0.55	0.57	0.526
rs2045028	17335	27944635	C/T			
rs1947384	25029	27952329	C/G	0.65	0.61	0.198
rs1947385	25111	27952411	C/T			
rs1901744	28811	27956111	A/G	0.19	0.18	0.674
rs1901745	28863	27956163	A/T	0.15	0.18	0.448
rs971882	30809	27958109	A/C			
rs1377338	40985	27968285	A/C	0.29	0.22	0.039
rs2305452	45147	27972447	C/T	0.31	0.26	0.067
rs2305451	45282	27972582	A/G	0.49	0.56	0.063
rs2123472	46168	27973468	G/T	0.42	0.49	0.039
rs2167768	46328	27973628	A/G	0.36	0.34	0.396
rs1563055	49077	27976377	A/G			
rs2290371	51925	27979225	C/T	0.28	0.23	0.054
rs2290370	52141	27979441	A/G	0.85	0.83	0.488
rs2290369	52168	27979468	C/T	0.41	0.49	0.036
rs2874904	60852	27988152	C/T	0.29	0.22	0.062
rs3213997	62468	27989768	A/G	0.44	0.50	0.064
rs3213998	65572	27992872	G/T	0.84	0.82	0.336
rs1530929	79089	28006389	A/C	0.48	0.52	0.311
rs1000275	79541	28006841	C/T	0.86	0.87	0.566
rs1000274	79790	28007090	C/T	0.54	0.59	0.159
rs3757896	90843	28018143	A/G			
rs3757895	90978	28018278	C/T	0.45	0.49	0.308
rs3757894	91052	28018352	C/G	0.09	0.09	0.914
rs3757893	91131	28018431	A/G	0.15	0.14	0.803
rs3757892	91132	28018432	C/T	0.09	0.08	0.798
rs3757891	94439	28021739	A/G			
rs3757890	94621	28021921	A/T	0.98	0.95	0.159

**TABLE 21**

dbSNP rs#	Position in SEQ ID NO: 2	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1000658	211	27927511	C/T	0.80	0.82	0.443
rs1984880	473	27927773	C/T	0.48	0.47	0.898
rs999112	1536	27928836	C/T	0.72	0.76	0.319
rs735880	5639	27932939	C/T	0.20	0.22	0.598
rs2045029	17186	27944486	A/G	0.52	0.54	0.581
rs2045028	17335	27944635	C/T			
rs1947384	25029	27952329	C/G	0.62	0.59	0.348
rs1947385	25111	27952411	C/T			
rs1901744	28811	27956111	A/G	0.18	0.18	0.928
rs1901745	28863	27956163	A/T	0.13	0.17	0.113
rs971882	30809	27958109	A/C			
rs1377338	40985	27968285	A/C	0.27	0.27	0.961
rs2305452	45147	27972447	C/T	0.32	0.30	0.673
rs2305451	45282	27972582	A/G	0.47	0.47	0.911
rs2123472	46168	27973468	G/T	0.41	0.38	0.348
rs2167768	46328	27973628	A/G	0.39	0.37	0.664
rs1563055	49077	27976377	A/G			
rs2290371	51925	27979225	C/T	0.28	0.25	0.403
rs2290370	52141	27979441	A/G	0.85	0.84	0.939
rs2290369	52168	27979468	C/T	0.46	0.44	0.712
rs2874904	60852	27988152	C/T	0.24	0.24	0.888
rs3213997	62468	27989768	A/G	0.45	0.43	0.752
rs3213998	65572	27992872	G/T	0.81	0.78	0.373
rs1530929	79089	28006389	A/C	0.46	0.43	0.445
rs1000275	79541	28006841	C/T	0.87	0.86	0.767
rs1000274	79790	28007090	C/T	0.54	0.51	0.394
rs3757896	90843	28018143	A/G			
rs3757895	90978	28018278	C/T	0.47	0.42	0.202
rs3757894	91052	28018352	C/G	0.07	0.09	0.478
rs3757893	91131	28018431	A/G	0.17	0.16	0.653
rs3757892	91132	28018432	C/T	0.09	0.07	0.567
rs3757891	94439	28021739	A/G			
rs3757890	94621	28021921	A/T	0.97	0.97	0.728

[0241] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1B for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1B can be determined by consulting Table 19. For example, the left-most X on the left graph is at position 27927511. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0242] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to

expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than  $10^{-8}$  were truncated at that value.

[0243] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is placed at the 3' end of each gene to show the direction of transcription.

#### Example 6

##### CHDC1 Region Proximal SNPs

[0244] It has been discovered that SNP rs912428 in calponin homology (CH) domain containing 1 (*CHDC1*) is associated with occurrence of osteoarthritis in subjects.

[0245] Forty-three additional allelic variants proximal to rs912428 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 22. The chromosome positions provided in column four of Table 22 are based on Genome "Build 34" of NCBI's GenBank.

**TABLE 22**

dbSNP rs#	Chromosome	Position in SEQ ID NO: 3	Chromosome Position	Allele Variants
rs1012628	13	243	44917643	c/t
rs1570976	13	10208	44927608	c/t
rs912436	13	15049	44932449	c/t
rs912435	13	15111	44932511	a/g
rs912433	13	15272	44932672	c/t
rs912432	13	15287	44932687	a/g
rs912431	13	15326	44932726	a/g
rs912430	13	15327	44932727	c/t
rs1408225	13	17038	44934438	c/t
rs998657	13	19391	44936791	a/g
rs1324006	13	21702	44939102	c/t
rs1924417	13	22431	44939831	c/g
rs2038728	13	22881	44940281	a/g

dbSNP rs#	Chromosome	Position in SEQ ID NO: 3	Chromosome Position	Allele Variants
rs912429	13	27744	44945144	a/t
rs3742269	13	32564	44949964	a/g
rs3742270	13	32698	44950098	a/c
rs3803192	13	33104	44950504	g/t
rs3803191	13	33181	44950581	c/t
rs754106	13	33256	44950656	c/t
rs2005053	13	33543	44950943	c/t
rs1535793	13	35567	44952967	c/t
rs1886220	13	40085	44957485	c/t
rs1886219	13	40482	44957882	a/t
rs1535792	13	45641	44963041	a/t
rs1535791	13	46059	44963459	a/g
rs912428	13	48504	44965904	c/t
rs1886218	13	48919	44966319	a/c
rs1570622	13	49693	44967093	c/t
rs912427	13	49874	44967274	a/g
rs912426	13	50020	44967420	a/g
rs3068693	13	50616	44968016	-/ttt
rs1570621	13	50719	44968119	a/g
rs1886965	13	55511	44972911	c/t
rs1008849	13	65533	44982933	a/g
rs912434	13	70529	44987929	a/c
rs3889095	13	75591	44992991	c/t
rs716223	13	77266	44994666	g/t
rs2897207	13	80368	44997768	g/t
rs1570620	13	82475	44999875	a/g
rs1467605	13	92462	45009862	g/t
rs1467604	13	92480	45009880	c/t
rs1408224	13	95819	45013219	c/t
rs1408223	13	96275	45013675	c/t

#### Assay for Verifying and Allelotyping SNPs

[0246] The methods used to verify and allelotype the 43 proximal SNPs of Table 22 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 23 and Table 24, respectively.

**TABLE 23**

dbSNP rs#	Forward PCR primer	Reverse PCR primer
Rs1012628	ACGTTGGATGGATTTTCTGTGTCCCCCAAG	ACGTTGGATGTTGCAACAGAGAGAGCTCTG
Rs1570976	ACGTTGGATGTGATGTGTCTGCTGTGTTGG	ACGTTGGATGTTACATGGCGAGGTCTTAG
rs912436	ACGTTGGATGCCATATAAGGTGGTTATGGG	ACGTTGGATGCAAAACAGTTTTTCTGAGGC
rs912435	ACGTTGGATGCAAGCCAATATCCAAGACAG	ACGTTGGATGAAAAACCTGTTTGTGAGGCC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs912433	ACGTTGGATGTGCCTTCCATCCTTAACACG	ACGTTGGATGGGCTTGAGCTTAGATATGGC
rs912432	ACGTTGGATGAAATAGTTGGGTTTTGTGCC	ACGTTGGATGATTTGGTGTAAATTGCAGTG
rs912431	ACGTTGGATGTGGAAGGCACAAAACCCAAC	ACGTTGGATGCAGAAGCTAGGCTTCCTATG
rs912430	ACGTTGGATGTGGAAGGCACAAAACCCAAC	ACGTTGGATGCAGAAGCTAGGCTTCCTATG
Rs1408225	ACGTTGGATGGGGCACCATGACAATATTCC	ACGTTGGATGACACCTTGATCTTGGACTTC
rs998657	ACGTTGGATGACTGGGCCAGGGAGGAATAG	ACGTTGGATGGTTGGGGAGATAATACAGAAG
Rs1324006	ACGTTGGATGGCTGAAAACCCAAATGTGTG	ACGTTGGATGCCAGCTATCAGCTCCATTTC
Rs1924417	ACGTTGGATGACAAAAGCAAGCCTTCACAG	ACGTTGGATGGTACTGTAAAAGGTACTGTG
Rs2038728	ACGTTGGATGAAGGCTTTTGGACACAAGTC	ACGTTGGATGGCACCTCTTATGATGTTCCC
rs912429	ACGTTGGATGTTCAATTCCCCAAAGCCCTC	ACGTTGGATGGGCAAGTTCATAACCTCTC
Rs3742269	ACGTTGGATGGAGAAAAGAGAACGAGAAGG	ACGTTGGATGTAAATGACAGCAGTCTGGAG
Rs3742270	ACGTTGGATGCTAAAACCAAAGCTGACGGG	ACGTTGGATGTTCTGCTCCTGTGGCATAGC
Rs3803192	ACGTTGGATGTCCTTTTGTCTCTGCGATGC	ACGTTGGATGTGCTTCCCCATCAGTTCTTG
Rs3803191	ACGTTGGATGCTGTCTGTACATTACCAGGC	ACGTTGGATGAATAGCAGCTGGAGGATCTC
rs754106	ACGTTGGATGTTCTTACCATCCAGCAAGGC	ACGTTGGATGGCCTGGTAATGTACAGACAG
Rs2005053	ACGTTGGATGCTGTTGCTAGCTTGGATTTG	ACGTTGGATGTTCCCTGTCTTTCTGGCAT
Rs1535793	ACGTTGGATGAACAAAGAGGAACAGAGCCC	ACGTTGGATGGCATAAGCCCCCTTTCTAG
Rs1886220	ACGTTGGATGTCACCGTGTTAGCGAGAATG	ACGTTGGATGTAATCCAGCACTTTGGGAG
Rs1886219	ACGTTGGATGTGTAAGTGGATTGCTGGAG	ACGTTGGATGTACATCAATAGCCGAGGAAG
Rs1535792	ACGTTGGATGCTGTATATCAGTGAAGTCTC	ACGTTGGATGCAGAGAAGAAACATCTCAGC
Rs1535791	ACGTTGGATGGAGGGTTTATCCTTACAATTG	ACGTTGGATGTTTTAGGGTCCCTTGATAAG
rs912428	ACGTTGGATGACTACATCCATTCCAGGGAG	ACGTTGGATGTCAGATCAGAGTGAGTTAG
Rs1886218	ACGTTGGATGTCCCGAAAACAAGTCAAGAC	ACGTTGGATGAGTCCAGGCAAAACAGTAAG
Rs1570622	ACGTTGGATGATAGCTGCCACACTCTTTAG	ACGTTGGATGGCGCAGTTTAGAAAAACCTG
rs912427	ACGTTGGATGTAGGGTTCTCGATGGGTATG	ACGTTGGATGTTTGGCCTGGTCACTTTAGG
rs912426	ACGTTGGATGTTAGAGGATGCATAGGCCAG	ACGTTGGATGAAGTCACTTACTGCATGGTC
Rs3068693	ACGTTGGATGAAATTGGCCACATGGAATCC	ACGTTGGATGCTACCTTTAACATCCCTGTC
Rs1570621	ACGTTGGATGAATTAAGAATGGCAGCTATG	ACGTTGGATGGTTTAAACTAAAAACAC
Rs1886965	ACGTTGGATGCTGCTAAGGATATGTGTTTCC	ACGTTGGATGACACCAGTGCTCAGTATTTG
Rs1008849	ACGTTGGATGGCAGTTGTGAATTGTGCAGC	ACGTTGGATGTGGTGCAGAACATGTCAGAC
rs912434	ACGTTGGATGTTCTGACATGTACAGACGTG	ACGTTGGATGTCCTGGGAAATCTTCCATC
Rs3889095	ACGTTGGATGAAGGTAATGATATGTCCCCC	ACGTTGGATGCGCATTTTACAGAGACATTG
rs716223	ACGTTGGATGACACTGTCTCTAGAAGCAGG	ACGTTGGATGGAAGCAGGAAAAGAGTGAGG
Rs2897207	ACGTTGGATGTCAGCCTCCAGAACTATGAG	ACGTTGGATGAACAGAGAGAGACCCTGTCT
Rs1570620	ACGTTGGATGCTGTTCTGCTTGTATATGG	ACGTTGGATGGAAGGAAGTCTATTACAGCCC
Rs1467605	ACGTTGGATGATGTTACAGGGTGGTAAGCG	ACGTTGGATGTAAAGTTGCCACGCTTCTCC
Rs1467604	ACGTTGGATGATATACGGCATGTTACAGGG	ACGTTGGATGTTAAAGTTGCCACGCTTCTC
Rs1408224	ACGTTGGATGACTTCCCACTCCTCTAGACA	ACGTTGGATGTATTGGCTGGGTAGCACTCC
Rs1408223	ACGTTGGATGTCATTACCAGTTCCACAGAG	ACGTTGGATGTTGAGACATCATGAGGAGTG

**TABLE 24**

dbSNP rs#	Extend Primer	Term Mix
Rs1012628	CTGTGTCCCCCAAGTCTTTG	ACG
Rs1570976	TTGGCATTCTTTGAGAA	ACT
rs912436	AGGTGGTTATGGGTTTGTCACTCA	ACT
rs912435	TCCAAAAAGCCCAAGAAATTCT	ACT
rs912433	CCTTAACACGTTTATAATAGATTA	ACG
rs912432	GTGCCTTCCATCCTTAACAC	ACT
rs912431	GGCACAAAACCCAACATTTTTTC	ACG
rs912430	GCACAAAACCCAACATTTTTTCC	ACT
Rs1408225	CCTCAGACTGGGTGGCTTA	ACT
rs998657	CACCCACCTGAGGGAGGC	ACT
Rs1324006	GATACCTTGAAGAATTTTTAAAAC	ACG
Rs1924417	TTTAGGCACATTTGTACTTATAAA	ACT
Rs2038728	TGGACACAAGTCCATGCAACA	ACG
rs912429	CTGTGACAGGTGCTATTATCA	CGT
Rs3742269	TTTTGGACCGATTTCGGTG	ACT
Rs3742270	GCTGACGGGGATTCCCTTTA	ACT
Rs3803192	GATGCACTAAAAGCAGCAATGT	ACT
Rs3803191	TCCAGCCTTCATATTTTCCTC	ACG
rs754106	ATCCAGCAAGGCACTTAGAAT	ACT
Rs2005053	TGTGGCCTTCAGATGCTTACAT	ACG
Rs1535793	GAGGAACAGAGCCCAAAGGACA	ACT
Rs1886220	CTGACCTCGTGATCCGCC	ACG
Rs1886219	ACTGGATTTGCTGGAGTTAAGAA	CGT
Rs1535792	TATCAGTGAAGTGCCTTTCTTTT	CGT
Rs1535791	TTATCCTTACAATTGAAGAAAGGA	ACT
rs912428	CCATTCCAGGGAGACTCCCA	ACT
Rs1886218	GAAAACAAGTCAAGACATTTATTG	ACT
Rs1570622	CTGCCACACTCTTTAGATGAAGTT	ACG
rs912427	GGGAGATGACAGAACAAACT	ACT
rs912426	AGGTGCCAAGTGTTAGAAGAAAC	ACG
Rs3068693	GCCTCACATTGTTTTTTTTTTTTT	ACT
Rs1570621	TCGGTCATAACTTTAATGAAGG	ACG
Rs1886965	TGATTTTATGACTCACATTATTC	ACT
Rs1008849	GTGAATTGTGCAGCTATAAACATG	ACG
rs912434	AGACGTGCCAGCTATGATA	ACT
Rs3889095	TCCCCATAACATTTTCAGCAT	ACT
rs716223	GTGGTTTGTATTTCCAGTGTCA	ACT
Rs2897207	AACTATGAGAAATAAATGTGTGGG	ACT
Rs1570620	TTGATATGGTTCTTGGTTGTTGG	ACG
Rs1467605	GTAAGCGCTAGAAAGAAAAATAA	ACT
Rs1467604	ACGGCATGTTACAGGGTGGTAAG	ACG
Rs1408224	GGGCACACATTCAGAAGTCCCC	ACG
Rs1408223	ACAGAGGAAGACCAAATGACA	ACG

# Genetic Analysis

[0247] Allelotyping results from the discovery cohort are shown for cases and controls in Table 25. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 (A1 AF = 1-A2 AF). For example, the SNP rs1570976 has the following case and control allele frequencies: case A1 (C) = 0.49; case A2 (T) = 0.51; control A1 (C) = 0.53; and control A2 (T) = 0.47, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

**TABLE 25**

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1012628	243	44917643	C/T	0.70	0.70	0.768
rs1570976	10208	44927608	C/T	0.51	0.47	0.125
rs912436	15049	44932449	C/T	0.98	untyped	
rs912435	15111	44932511	A/G	0.64	0.36	<b>~0.0001</b>
rs912433	15272	44932672	C/T	0.22	0.23	0.581
rs912432	15287	44932687	A/G	0.46	0.44	0.282
rs912431	15326	44932726	A/G	0.46	0.46	0.969
rs912430	15327	44932727	C/T	0.20	0.19	0.584
rs1408225	17038	44934438	C/T			
rs998657	19391	44936791	A/G	0.47	0.44	0.254
rs1324006	21702	44939102	C/T	0.55	0.53	0.419
rs1924417	22431	44939831	C/G	0.53	0.49	0.108
rs2038728	22881	44940281	A/G	0.34	0.38	0.082
rs912429	27744	44945144	A/T			
rs3742269	32564	44949964	A/G	0.83	0.83	0.967
rs3742270	32698	44950098	A/C	0.53	0.50	0.170
rs3803192	33104	44950504	G/T			
rs3803191	33181	44950581	C/T			
rs754106	33256	44950656	C/T	0.40	0.41	0.714
rs2005053	33543	44950943	C/T	0.40	0.40	0.877
rs1535793	35567	44952967	C/T	0.26	0.26	0.910
rs1886220	40085	44957485	C/T			
rs1886219	40482	44957882	A/T	0.21	0.22	0.867
rs1535792	45641	44963041	A/T	0.73	0.71	0.550
rs1535791	46059	44963459	A/G	0.08	0.15	<b>0.009</b>
rs912428	48504	44965904	C/T			
rs1886218	48919	44966319	A/C			
rs1570622	49693	44967093	C/T	0.73	0.75	0.451
rs912427	49874	44967274	A/G	0.68	0.70	0.352
rs912426	50020	44967420	A/G	0.76	0.77	0.680
rs3068693	50616	44968016	-/TTT	0.22	0.21	0.597
rs1570621	50719	44968119	A/G	0.19	0.18	0.569
rs1886965	55511	44972911	C/T			
rs1008849	65533	44982933	A/G	0.48	0.43	0.160
rs912434	70529	44987929	A/C	0.23	0.23	0.988
rs3889095	75591	44992991	C/T	0.90	0.90	0.880
rs716223	77266	44994666	G/T	0.91	0.90	0.981
rs2897207	80368	44997768	G/T	0.46	0.46	0.921
rs1570620	82475	44999875	A/G	0.67	0.68	0.738
rs1467605	92462	45009862	G/T	0.29	0.22	<b>0.044</b>

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1467604	92480	45009880	C/T	0.68	0.67	0.537
rs1408224	95819	45013219	C/T	0.66	0.65	0.683
rs1408223	96275	45013675	C/T	0.29	0.28	0.587

[0248] The *CHDC1* proximal SNPs were also allelotyped in the replication cohorts using the methods described herein and the primers provided in Tables 23 and 24. The replication allelotyping results for replication cohort #1 and replication cohort #2 are provided in Tables 26 and 27, respectively.

**TABLE 26**

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1012628	243	44917643	C/T	0.69	0.72	0.337
rs1570976	10208	44927608	C/T	0.48	0.46	0.490
rs912436	15049	44932449	C/T			
rs912435	15111	44932511	A/G	0.16	untyped	0.637
rs912433	15272	44932672	C/T	0.28	0.28	0.984
rs912432	15287	44932687	A/G	0.46	0.42	0.260
rs912431	15326	44932726	A/G	0.46	0.48	0.602
rs912430	15327	44932727	C/T	0.18	0.20	0.476
rs1408225	17038	44934438	C/T			
rs998657	19391	44936791	A/G	0.46	0.43	0.380
rs1324006	21702	44939102	C/T	0.54	0.53	0.811
rs1924417	22431	44939831	C/G	0.51	0.49	0.440
rs2038728	22881	44940281	A/G	0.35	0.39	0.181
rs912429	27744	44945144	A/T			
rs3742269	32564	44949964	A/G	0.84	0.85	0.911
rs3742270	32698	44950098	A/C	0.56	0.50	0.090
rs3803192	33104	44950504	G/T			
rs3803191	33181	44950581	C/T			
rs754106	33256	44950656	C/T	0.40	0.40	0.827
rs2005053	33543	44950943	C/T	0.40	0.37	0.328
rs1535793	35567	44952967	C/T	0.27	0.24	0.259
rs1886220	40085	44957485	C/T			
rs1886219	40482	44957882	A/T	0.22	0.19	0.302
rs1535792	45641	44963041	A/T	0.73	0.76	0.435
rs1535791	46059	44963459	A/G	0.08	0.08	0.958
rs912428	48504	44965904	C/T			
rs1886218	48919	44966319	A/C			
rs1570622	49693	44967093	C/T	0.71	0.79	0.007
rs912427	49874	44967274	A/G	0.65	0.73	0.007
rs912426	50020	44967420	A/G	0.74	0.80	0.047
rs3068693	50616	44968016	-/TTT	0.25	0.21	0.236
rs1570621	50719	44968119	A/G	0.22	0.15	0.028
rs1886965	55511	44972911	C/T			
rs1008849	65533	44982933	A/G	0.47	untyped	NA
rs912434	70529	44987929	A/C	0.24	0.19	0.083
rs3889095	75591	44992991	C/T	0.91	0.91	0.867
rs716223	77266	44994666	G/T	0.91	0.93	0.598
rs2897207	80368	44997768	G/T	0.48	0.45	0.321
rs1570620	82475	44999875	A/G	0.66	0.72	0.034
rs1467605	92462	45009862	G/T	0.29	0.22	0.044
rs1467604	92480	45009880	C/T	0.66	0.70	0.307

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1408224	95819	45013219	C/T	0.64	0.67	0.312
rs1408223	96275	45013675	C/T	0.31	0.23	<b>0.028</b>

TABLE 27

dbSNP rs#	Position in SEQ ID NO: 3	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1012628	243	44917643	C/T	0.71	0.68	0.438
rs1570976	10208	44927608	C/T	0.55	0.50	0.159
rs912436	15049	44932449	C/T			
rs912435	15111	44932511	A/G	0.66	untyped	
rs912433	15272	44932672	C/T	0.14	0.17	0.479
rs912432	15287	44932687	A/G	0.47	0.46	0.806
rs912431	15326	44932726	A/G	0.46	0.44	0.513
rs912430	15327	44932727	C/T	0.23	0.17	0.084
rs1408225	17038	44934438	C/T			
rs998657	19391	44936791	A/G	0.48	0.45	0.518
rs1324006	21702	44939102	C/T	0.55	0.52	0.324
rs1924417	22431	44939831	C/G	0.54	0.49	0.123
rs2038728	22881	44940281	A/G	0.34	0.37	0.295
rs912429	27744	44945144	A/T			
rs3742269	32564	44949964	A/G	0.82	0.82	0.861
rs3742270	32698	44950098	A/C	0.50	0.49	0.873
rs3803192	33104	44950504	G/T			
rs3803191	33181	44950581	C/T			
rs754106	33256	44950656	C/T	0.41	0.44	0.346
rs2005053	33543	44950943	C/T	0.40	0.44	0.302
rs1535793	35567	44952967	C/T	0.25	0.31	0.096
rs1886220	40085	44957485	C/T			
rs1886219	40482	44957882	A/T	0.20	0.27	0.053
rs1535792	45641	44963041	A/T	0.73	0.63	<b>0.007</b>
rs1535791	46059	44963459	A/G	NA	0.27	NA
rs912428	48504	44965904	C/T			
rs1886218	48919	44966319	A/C			
rs1570622	49693	44967093	C/T	0.75	0.67	<b>0.040</b>
rs912427	49874	44967274	A/G	0.71	0.64	0.059
rs912426	50020	44967420	A/G	0.78	0.72	0.065
rs3068693	50616	44968016	-/TTT	0.19	0.21	0.520
rs1570621	50719	44968119	A/G	0.15	0.21	0.077
rs1886965	55511	44972911	C/T			
rs1008849	65533	44982933	A/G	0.49	0.43	0.138
rs912434	70529	44987929	A/C	0.21	0.28	<b>0.027</b>
rs3889095	75591	44992991	C/T	0.89	0.88	0.583
rs716223	77266	44994666	G/T	0.90	0.87	0.368
rs2897207	80368	44997768	G/T	0.44	0.48	0.276
rs1570620	82475	44999875	A/G	0.70	0.62	<b>0.026</b>
rs1467605	92462	45009862	G/T			
rs1467604	92480	45009880	C/T	0.71	0.62	<b>0.018</b>
rs1408224	95819	45013219	C/T	0.68	0.61	0.060
rs1408223	96275	45013675	C/T	0.27	0.34	<b>0.023</b>

[0249] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values

were plotted in Figure 1C for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1C can be determined by consulting Table 25. For example, the left-most X on the left graph is at position 44917643. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0250] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than  $10^{-8}$  were truncated at that value.

[0251] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is placed at the 3' end of each gene to show the direction of transcription.

#### Example 7

##### ERG Region Proximal SNPs

[0252] It has been discovered that SNP rs1888475 in v-ets erythroblastosis virus E26 oncogene like (*ERG*) is associated with occurrence of osteoarthritis in subjects. One hundred sixty-six additional allelic variants proximal to rs1888475 were identified and subsequently allelotyped in osteoarthritis case and control sample sets as described in Examples 1 and 2. The polymorphic variants are set forth in Table 28. The chromosome positions provided in column four of Table 28 are based on Genome "Build 34" of NCBI's GenBank.

**TABLE 28**

dbSNP rs#	Chromosome	Position in SEQ ID NO: 4	Chromosome Position	Allele Variants
rs2898353	21	231	38783681	a/t
rs960818	21	882	38784332	a/g
rs960819	21	960	38784410	a/c
rs2410034	21	1194	38784644	a/c
rs2836437	21	1530	38784980	a/g
rs2836438	21	1673	38785123	a/g
rs2836439	21	2096	38785546	c/t
rs2836440	21	2285	38785735	a/g
rs2226683	21	5873	38789323	c/t
rs2836441	21	7256	38790706	a/g
rs2836442	21	7988	38791438	a/g
rs2836443	21	8222	38791672	g/t
rs2836444	21	8381	38791831	c/t
rs3787906	21	8814	38792264	c/t
rs3838108	21	8915	38792365	-/c
rs2836445	21	9642	38793092	a/g
rs2836446	21	9902	38793352	a/t
rs3787908	21	10619	38794069	a/g
rs2836447	21	10927	38794377	c/t
rs2836448	21	11032	38794482	c/t
rs2836450	21	14377	38797827	c/t
rs2836451	21	15608	38799058	c/t
rs1015022	21	15928	38799378	c/g
rs2836452	21	16296	38799746	a/g
rs2836453	21	17598	38801048	a/t
rs3787909	21	19272	38802722	a/g
rs2836454	21	20084	38803534	a/g
rs2836455	21	20577	38804027	a/t
rs2155718	21	28051	38811501	a/g
rs2836456	21	29466	38812916	a/g
rs2836457	21	29530	38812980	c/t
rs2836458	21	29987	38813437	a/g
rs2032323	21	30012	38813462	c/t
rs2051400	21	30322	38813772	g/t
rs2836459	21	32216	38815666	c/t
rs2836460	21	32516	38815966	c/t
rs2836461	21	32544	38815994	a/g
rs2836462	21	32746	38816196	a/g
rs2836463	21	33137	38816587	g/t
rs2836464	21	33538	38816988	a/g
rs2836465	21	33798	38817248	c/t
rs2836466	21	33802	38817252	a/c
rs2836467	21	33964	38817414	c/t
rs3827204	21	34132	38817582	a/g
rs2836468	21	34210	38817660	c/t
rs3787911	21	34317	38817767	a/g

dbSNP rs#	Chromosome	Position in SEQ ID NO: 4	Chromosome Position	Allele Variants
rs2836469	21	34499	38817949	c/t
rs2836470	21	34753	38818203	a/c
rs2212599	21	34845	38818295	c/t
rs2836472	21	35335	38818785	c/t
rs2836473	21	36423	38819873	c/t
rs1888469	21	36450	38819900	a/g
rs1888470	21	36481	38819931	g/t
rs2032322	21	38447	38821897	c/g
rs2410035	21	38784	38822234	c/t
rs1573332	21	39387	38822837	a/t
rs2836474	21	39458	38822908	c/t
rs2836475	21	39822	38823272	c/g
rs3787914	21	40305	38823755	c/g
rs1888471	21	40869	38824319	c/t
rs1888472	21	40926	38824376	c/t
rs1888473	21	41010	38824460	c/t
rs1888474	21	41134	38824584	c/t
rs2836476	21	41984	38825434	a/g
rs3787916	21	42172	38825622	a/t
rs2836477	21	42753	38826203	g/t
rs970043	21	43011	38826461	c/t
rs2212600	21	43176	38826626	a/g
rs2836478	21	43320	38826770	g/t
rs2836479	21	43381	38826831	a/t
rs1475877	21	44142	38827592	a/g
rs2836480	21	44383	38827833	a/g
rs2836481	21	44726	38828176	c/t
rs2836483	21	45087	38828537	a/g
rs2836484	21	45141	38828591	c/t
rs2836485	21	45359	38828809	c/g
rs2836486	21	45421	38828871	c/t
rs2836487	21	45456	38828906	c/t
rs1893199	21	45467	38828917	c/t
rs2836488	21	45486	38828936	c/t
rs1893200	21	45709	38829159	a/g
rs1893201	21	45716	38829166	a/g
rs2836489	21	47626	38831076	c/t
rs1888475	21	49413	38832863	a/g
rs2836490	21	49796	38833246	c/t
rs2836491	21	49962	38833412	a/g
rs2836492	21	50075	38833525	c/t
rs2836493	21	50093	38833543	a/g
rs2836494	21	50571	38834021	c/t
rs2836495	21	50615	38834065	a/g
rs2898354	21	50780	38834230	a/g
rs3065390	21	50851	38834301	-/ta
rs2836496	21	51459	38834909	a/c

dbSNP rs#	Chromosome	Position in SEQ ID NO: 4	Chromosome Position	Allele Variants
rs2836497	21	53193	38836643	c/t
rs2836498	21	53702	38837152	c/t
rs2836499	21	53736	38837186	a/c
rs2836500	21	53795	38837245	c/t
rs2836501	21	54109	38837559	a/t
rs2836502	21	54126	38837576	c/t
rs2836503	21	54230	38837680	a/c
rs2836504	21	54894	38838344	c/t
rs3787917	21	55455	38838905	a/g
rs2836505	21	55499	38838949	a/g
rs2836506	21	56522	38839972	c/t
rs2836507	21	56662	38840112	c/t
rs2836508	21	56954	38840404	a/g
rs2836509	21	57267	38840717	a/g
rs2836510	21	58282	38841732	a/g
rs2836511	21	58916	38842366	a/c
rs2212601	21	59544	38842994	c/g
rs2212602	21	59666	38843116	c/t
rs2226682	21	59913	38843363	a/t
rs2836512	21	66846	38850296	a/g
rs2836513	21	67245	38850695	g/t
rs1999328	21	67652	38851102	a/c
rs2212603	21	67955	38851405	a/g
rs3787919	21	67966	38851416	a/c
rs2836514	21	68420	38851870	a/g
rs1023153	21	70226	38853676	a/g
rs1023372	21	70810	38854260	c/t
rs2212604	21	72246	38855696	a/g
rs2226684	21	73330	38856780	g/t
rs2212605	21	73457	38856907	c/t
rs2187307	21	74389	38857839	a/g
rs3065412	21	74638	38858088	-/aa
rs2898355	21	74640	38858090	a/c
rs2836518	21	75358	38858808	a/c
rs3838110	21	75952	38859402	-/g
rs2836519	21	76098	38859548	a/g
rs3827207	21	77836	38861286	a/g
rs2836520	21	78449	38861899	a/c
rs2836521	21	78507	38861957	g/t
rs2836522	21	80031	38863481	g/t
rs2836523	21	81695	38865145	c/t
rs2836524	21	82775	38866225	a/g
rs2836525	21	82795	38866245	a/g
rs3833350	21	84611	38868061	-/c
rs2836526	21	84657	38868107	c/t
rs2836527	21	84693	38868143	a/c
rs3834676	21	85020	38868470	-/t

dbSNP rs#	Chromosome	Position in SEQ ID NO: 4	Chromosome Position	Allele Variants
rs2836528	21	85048	38868498	c/t
rs3761364	21	85100	38868550	c/t
rs2836529	21	85325	38868775	a/c
rs2836530	21	85452	38868902	c/t
rs3761366	21	85868	38869318	a/g
rs2836531	21	85936	38869386	a/g
rs2836532	21	85990	38869440	a/t
rs2836533	21	86139	38869589	c/t
rs2836534	21	86497	38869947	c/t
rs2836535	21	87236	38870686	a/g
rs2836536	21	87248	38870698	c/t
rs3827208	21	87533	38870983	c/g
rs715860	21	87912	38871362	a/g
rs717231	21	88108	38871558	g/t
rs2836537	21	88494	38871944	a/c
rs2836538	21	89598	38873048	a/c
rs2836539	21	90235	38873685	a/t
rs2836540	21	91287	38874737	g/t
rs2836541	21	91359	38874809	c/t
rs2836542	21	92384	38875834	a/c
rs2836543	21	92410	38875860	c/t
rs881837	21	92900	38876350	c/t
rs3949052	21	94495	38877945	a/g
rs2065307	21	94512	38877962	a/g
rs3216105	21	97777	38881227	-/a
rs2073427	21	98333	38881783	c/t

#### Assay for Verifying and Allelotyping SNPs

[0253] The methods used to verify and allelotype the 166 proximal SNPs of Table 28 are the same methods described in Examples 1 and 2 herein. The primers and probes used in these assays are provided in Table 29 and Table 30, respectively.

**TABLE 29**

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs2898353	ACGTTGGATGAATGTGAATGTGGAGGTAGC	ACGTTGGATGCTCCCTTGCTGGTTTTTTTG
rs960818	ACGTTGGATGTGGGATTTTTCCCAGAAGAG	ACGTTGGATGCTGTGCAGAGAAACATGATG
rs960819	ACGTTGGATGCTGTCTCCCTTCTCTTTATC	ACGTTGGATGCATCATGTTTCTCTGCACAG
rs2410034	ACGTTGGATGTTTAGAGACATTTCTCCTAG	ACGTTGGATGTTAGGATGATGTTAGTTTGG
rs2836437	ACGTTGGATGAGCTTCTGCGATATCAGTGG	ACGTTGGATGTTCTGTGCAGCACATTCTCC
rs2836438	ACGTTGGATGAACATGTCTTGCCAAGCTC	ACGTTGGATGCCACTGTGACCTCTGGATTT
rs2836439	ACGTTGGATGCCTAGTGTATAAAGTGATGC	ACGTTGGATGTCCTTTCTAGGCACCAATAC
rs2836440	ACGTTGGATGAGATCCTAACCAACCACAGC	ACGTTGGATGAGGTAGGTAGATACAAGGCC
rs2226683	ACGTTGGATGAATATGGCTCCTATAGACAG	ACGTTGGATGTTTTGGGTCACAAAATCAAG

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs2836441	ACGTTGGATGTTACCTTAATAGTGCTGGCC	ACGTTGGATGACTTTCTGGTCAGAGAGAAG
rs2836442	ACGTTGGATGCAAGGACTCTAGGCTTACAG	ACGTTGGATGGGGACATTTGTAGTCACTTC
rs2836443	ACGTTGGATGGGGCCCCATTACATGTCTAA	ACGTTGGATGTTGCTGTACTTCTTCGAG
rs2836444	ACGTTGGATGCTGCAACCAGGAATTGTCAG	ACGTTGGATGAGGACCATAAAGAGGTGTG
rs3787906	ACGTTGGATGTGAAAAGAGCGGAAATCAAC	ACGTTGGATGGTAAGAAAATCATTCTGTGG
rs3838108	ACGTTGGATGATGAATAAGATGGCAGGCTG	ACGTTGGATGAAGCTGCCAGATAAAACAG
rs2836445	ACGTTGGATGCATTTCCAAAATTAGACGCAG	ACGTTGGATGAAAAAGAGAAAAACAGATGC
rs2836446	ACGTTGGATGGTGCCTTGTCTATCAAGAG	ACGTTGGATGAGCATCCAAGCCTGGTAATC
rs3787908	ACGTTGGATGAATCACCACACTAGACCAGC	ACGTTGGATGCATGCAAGGGAAATGTGTGC
rs2836447	ACGTTGGATGATCTCCTCTCTTGTCTGCTG	ACGTTGGATGGAGGAAGGTTAGGAGCTAAG
rs2836448	ACGTTGGATGTGTAGGGATGTATAGGGCAG	ACGTTGGATGAAAGAGAGGAGATCCGTCTG
rs2836450	ACGTTGGATGTGTGGGCATCAGATGACAAC	ACGTTGGATGATCCCGTTAAATGCACCGAC
rs2836451	ACGTTGGATGCAGACAAACAACGTGACCC	ACGTTGGATGGTATTTCTTTTCTCGCCGC
rs1015022	ACGTTGGATGTCGAGCCAGCGCTTTTATC	ACGTTGGATGGTAACAGTCGTACATTCCGG
rs2836452	ACGTTGGATGATCACTGACACAGTCATGAG	ACGTTGGATGCCAGTAACTTTGCAGGTTTG
rs2836453	ACGTTGGATGTGTATTTCCCAAGATGGCCC	ACGTTGGATGCCTCACTTTCTGATGGAAGC
rs3787909	ACGTTGGATGACTTCTCAGTGTTCTGGCTG	ACGTTGGATGCGTCACTCTCTGTTTCATGG
rs2836454	ACGTTGGATGAGGAATGATTACAACTCC	ACGTTGGATGGAATGTTCAAATGTAGGGTGG
rs2836455	ACGTTGGATGGGTCTATTGCTGTGACATTT	ACGTTGGATGCATCCCAATTTTAAAGCAAG
rs2155718	ACGTTGGATGAGAACTCTCACACACAGCTG	ACGTTGGATGTGCCTCTTATTACAGCCCTG
rs2836456	ACGTTGGATGGGGATTGTCTGATCTCCTTG	ACGTTGGATGCCAGCTTTCCTTGTGCATG
rs2836457	ACGTTGGATGAACTCCTGGAATGAGTCACC	ACGTTGGATGATGCACAAAGGAAAGCTGGG
rs2836458	ACGTTGGATGATCACTTAGAAGCCCAGCAG	ACGTTGGATGTGATGCACACTCACTGAAGC
rs2032323	ACGTTGGATGGTAGCCGCACTTTGAGATGC	ACGTTGGATGAGCACAGAGTCGAGGAGGAG
rs2051400	ACGTTGGATGACAGACCTCAGACCAAAGTC	ACGTTGGATGTTTGTCTAGAGTAACCCCC
rs2836459	ACGTTGGATGGCAAGAATGTTACTTTCTGG	ACGTTGGATGCCATCAAATAGTTGGTTGTC
rs2836460	ACGTTGGATGCAATATCTGAGTTTACCCC	ACGTTGGATGGTAGATGAGAATCCGTGTG
rs2836461	ACGTTGGATGGTTACCCACACGGAATTCTC	ACGTTGGATGCCAGATCCAGGTTCTTTCTG
rs2836462	ACGTTGGATGTCTCCTCCGTATGTCTCCAT	ACGTTGGATGATCCCGGAACTCTCTGTTTC
rs2836463	ACGTTGGATGGCACTATTTGACTTGAGCTC	ACGTTGGATGAATTCAAGCCAGAAAGGCTC
rs2836464	ACGTTGGATGGTCTTTTTACCCCCAGTAAAG	ACGTTGGATGATAAGCAAAGGACCTTTTG
rs2836465	ACGTTGGATGTGAGCTCTTGTTTGGCCC	ACGTTGGATGGAGAATTCTCCAGCCTTCTC
rs2836466	ACGTTGGATGTGAGCTCTTGTTTGGCCC	ACGTTGGATGGAGAATTCTCCAGCCTTCTC
rs2836467	ACGTTGGATGGACTCTGCTCATTTCTTGG	ACGTTGGATGAAGAGTAGGGGTAGATGCAG
rs3827204	ACGTTGGATGTGAAGATCACACGTGGTGTA	ACGTTGGATGGGGTGAATGCCAAAAGAGG
rs2836468	ACGTTGGATGTAGAGGCAGGAAAGAGCATG	ACGTTGGATGTTTTGGCATTACCCTCTC
rs3787911	ACGTTGGATGTAACCTCTTCTGGATTCCG	ACGTTGGATGTCATGTGCTCTGAGAGCATC
rs2836469	ACGTTGGATGATTTCTCTACCTCATCCCC	ACGTTGGATGGGTTGAAGTCACGTAACAGC
rs2836470	ACGTTGGATGCCACTGTTAATCGTATTGCC	ACGTTGGATGACGGAAGGAAAGCCAAATGG
rs2212599	ACGTTGGATGAGGAGTTATTCTTCCCAAC	ACGTTGGATGCAGTGGTCCATTAAGAATCC
rs2836472	ACGTTGGATGGAGTATCGTTCTCTATCATG	ACGTTGGATGTAAAAGAGTCAGAGCAGGAC
rs2836473	ACGTTGGATGTCTCAGCCAGAGTTTTGACC	ACGTTGGATGAATCAACGCCTCTCTTCAG
rs1888469	ACGTTGGATGACCACCAGGAAGGGTCTGAA	ACGTTGGATGGAGGATCAGAGGCAGAAAAC
rs1888470	ACGTTGGATGGCGTTGATTGCAGTTTTCTG	ACGTTGGATGTTCTTTGGCCTCCGTGTAAG
rs2032322	ACGTTGGATGTGATACTCTGTTGAGCCTCC	ACGTTGGATGGGGGAGCAGTGATGAGTTAT
rs2410035	ACGTTGGATGAATCACTTGAACCCAGGAGG	ACGTTGGATGTTTTGAGACGGAGTTTCGC
rs1573332	ACGTTGGATGGGGTGAACTTTACAGAGAGG	ACGTTGGATGCTGCCAGACAGTTTGTAGAC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs2836474	ACGTTGGATGAATTCTGCACAGGAGAGTCC	ACGTTGGATGCAGGAAATGAAGATGTCGCC
rs2836475	ACGTTGGATGAGTTCTACATGGGAAGCTGC	ACGTTGGATGATATCTGTGTCTACAGGCC
rs3787914	ACGTTGGATGGGCTGAAGGCTAAAATCACC	ACGTTGGATGGTCTGAGAAGTAGGAATGGC
rs1888471	ACGTTGGATGACTGAGGCAATTGTGTAGAC	ACGTTGGATGTTGACTTTGTTTTGAGAGGC
rs1888472	ACGTTGGATGTTGCCTCTCAAAACAAAGTC	ACGTTGGATGCTATTATTCTGGAAGCAGCC
rs1888473	ACGTTGGATGAGAAAGTTCAGTTCTCAGCC	ACGTTGGATGTGTTTGCTCCTGTGAGTAAC
rs1888474	ACGTTGGATGTGTTATGTGAGTCCAGGGTG	ACGTTGGATGTCTTGTTATGTGGGTGGGTG
rs2836476	ACGTTGGATGTTACCTGTGACCTCATTTGG	ACGTTGGATGGAACACACAACATACGGTAC
rs3787916	ACGTTGGATGAAGGCATCTCAGTCATTCTC	ACGTTGGATGTGAGTTTGACACAAAGAAGC
rs2836477	ACGTTGGATGTTTAGCTCTCCTGGATGATG	ACGTTGGATGCCATGATTAGTGCATGAAGG
rs970043	ACGTTGGATGTATAACTCCCCTCTCTCCTG	ACGTTGGATGAGAGCAGACCCCTTATCAGAG
rs2212600	ACGTTGGATGGAAACAGGTGTTCAATTTGGC	ACGTTGGATGTCTGCATGAACCAGTAAGTC
rs2836478	ACGTTGGATGAGCTATTGAGTGTCACTTGC	ACGTTGGATGCAGAAGCTTCTGACTTCAAC
rs2836479	ACGTTGGATGAGTAGCCATCCTAATAGGTG	ACGTTGGATGAGCAAGTGACACTCAATAGC
rs1475877	ACGTTGGATGAATCAACACTCCCCGTGTTTC	ACGTTGGATGGGTACCTAGAGTAGTCCAAG
rs2836480	ACGTTGGATGTACCAAACCCACTGTACATC	ACGTTGGATGCATAACCTAACACATTGTGGG
rs2836481	ACGTTGGATGTAAGAAGTTCTTTCTCCCCC	ACGTTGGATGGCTGCTTCTTTTATAAGAGG
rs2836483	ACGTTGGATGCACTGAGGTAATCTCCAACC	ACGTTGGATGGGTGGAGATATGGCTTGATG
rs2836484	ACGTTGGATGAAGCCCACCAGAGTCATCAA	ACGTTGGATGACTACTGACCAGCTTTCCAG
rs2836485	ACGTTGGATGTTCTAAGTGAAGCCCTCCTC	ACGTTGGATGTACAGCTGTGCAAACAGTTG
rs2836486	ACGTTGGATGCATGGTCTGTTGCCTCTAAG	ACGTTGGATGCCCTAGCATTTTATGCATCC
rs2836487	ACGTTGGATGTGAATACCCACTAGGTCTCG	ACGTTGGATGCCACCCTAACTTAGAGGC
rs1893199	ACGTTGGATGGGCAACAGACCATGGTTTTG	ACGTTGGATGCTTCCCTTCAACATGCACTG
rs2836488	ACGTTGGATGGGCAACAGACCATGGTTTTG	ACGTTGGATGCTTCCCTTCAACATGCACTG
rs1893200	ACGTTGGATGAGTTAAGTCTTCGCATAACC	ACGTTGGATGCCTCTCACACACTAAATCTTG
rs1893201	ACGTTGGATGGTCTTCGCATAACCAAACAG	ACGTTGGATGCCTCTCACACACTAAATCTTG
rs2836489	ACGTTGGATGGTCAACCATGGAGCTTGAAC	ACGTTGGATGAGAAGACATGTGGGCTTGTG
rs1888475	ACGTTGGATGACCCCTGGCAAGTGAATTAC	ACGTTGGATGGGGAGGTGGATGTTCTTATC
rs2836490	ACGTTGGATGAAAGGCAGAGCTAAAGCAAG	ACGTTGGATGAGCACAACCCAGCAATGCAG
rs2836491	ACGTTGGATGACAACCTTGGAGTGGAAGGG	ACGTTGGATGATCCAGATGGATTCCACAGC
rs2836492	ACGTTGGATGACATATGGGCATGGAAGAGC	ACGTTGGATGAATCCATCTGGATGGAAGAC
rs2836493	ACGTTGGATGTTAAGAGTTCGGATGCTTGC	ACGTTGGATGGTAATCTGGACTTCTCTTCC
rs2836494	ACGTTGGATGGTGCATTCAATTTGAATTGCTG	ACGTTGGATGCAGTCTTACTTAAACTGAC
rs2836495	ACGTTGGATGGAATTTAACGAACTTCAGC	ACGTTGGATGGGATATTTTCAGGATATCTG
rs2898354	ACGTTGGATGTGTAACAAACCTGCACATCC	ACGTTGGATGGGTACTTTCAAATATCTGC
rs3065390	ACGTTGGATGCGAGACTCCATCTCAAAAAG	ACGTTGGATGTGGAAAGTACCAATAGCTTC
rs2836496	ACGTTGGATGTGGAGCTTAATGTGTTCTCTG	ACGTTGGATGGTTAGCCATGCATAAGACAG
rs2836497	ACGTTGGATGAGCCGGGATGACTGCTAGAC	ACGTTGGATGAGATGAGGCTGAAGAAGTAA
rs2836498	ACGTTGGATGGGTCCTGGGAAAATAGGATG	ACGTTGGATGCACCCCTTGCTCTTTCTGAAG
rs2836499	ACGTTGGATGACTAGTCAGAGCACAGTGAG	ACGTTGGATGGCTCTCTCCTTCTTTGACTC
rs2836500	ACGTTGGATGGCTTCTCTGGTTAGTAAGAGG	ACGTTGGATGATCAACTCAGGGCTCTTCTC
rs2836501	ACGTTGGATGACTCACAAAGGTTGACCTTG	ACGTTGGATGGAGGTCCAGGTTGAAAGAAC
rs2836502	ACGTTGGATGGAGGTCCAGGTTGAAAGAAC	ACGTTGGATGACTCACAAAGGTTGACCTTG
rs2836503	ACGTTGGATGGAGCAATTATCAACCCTACG	ACGTTGGATGATTCTCCCCCTTCACTCTTG
rs2836504	ACGTTGGATGGAGTCTGGGTATGGAAGAG	ACGTTGGATGTTCTCTAGAAATGGTGTCTGC
rs3787917	ACGTTGGATGTTTGGAGGAGGAATGCCTTG	ACGTTGGATGCGCCACAAACCTAAGAGAA
rs2836505	ACGTTGGATGTTTTGACTGCTCCACTCTG	ACGTTGGATGGCTCTCCCTCATTTGTTCTTC

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs2836506	ACGTTGGATGGGCTAAGGGCATCATTTTATC	ACGTTGGATGGTTTGCTGATTCATGGATGC
rs2836507	ACGTTGGATGAGCAAAGGTTCTGGTGTGG	ACGTTGGATGAAATGATGCCCTTAGCCCAG
rs2836508	ACGTTGGATGGTGTGATGATATTTTTCTCC	ACGTTGGATGTTTCAGGTATTCTCTTTGC
rs2836509	ACGTTGGATGTAAGCTTTCTAAGTCAATG	ACGTTGGATGTCATATGATAATGGTCTCTG
rs2836510	ACGTTGGATGCAGGGAGAGATCTAAACAGC	ACGTTGGATGGCCAAAGCTATAACACGTGG
rs2836511	ACGTTGGATGAGAACCTGACTTTTGGAGTG	ACGTTGGATGCTTCCTCATTGGTCAGAGTC
rs2212601	ACGTTGGATGCCAGCCTTTAGAACTGTGAG	ACGTTGGATGTGGGCTGCTGTAACAAAGTG
rs2212602	ACGTTGGATGACTACAACCAGCCAGAGATG	ACGTTGGATGCACAAACCTTGTGTGAACCC
rs2226682	ACGTTGGATGCCAAGATTGAACCAGGAAAG	ACGTTGGATGCACAAAAGAATTGAGGAGGTG
rs2836512	ACGTTGGATGCCCCAAAACCTTAGCATCCTG	ACGTTGGATGTGTTCTCCCTGCACTTCAAC
rs2836513	ACGTTGGATGCACTGGGGTTAGCAAGAAAC	ACGTTGGATGGACTGTGATTCACCCTGTCT
rs1999328	ACGTTGGATGAGTTACAGCGCAAATTGAGG	ACGTTGGATGGCCTTTATGACTCCATTTCTC
rs2212603	ACGTTGGATGTGGAGGGTGTCTGTGAGTAC	ACGTTGGATGTCATGGAGCAAGGTCTGTGG
rs3787919	ACGTTGGATGCCATCAGCTAGGATTCATGG	ACGTTGGATGTCTGTGAGTACCCACAATG
rs2836514	ACGTTGGATGCAGGTCTAACTAACTGATGAC	ACGTTGGATGGCCTCTACTGTTATTTAAGG
rs1023153	ACGTTGGATGTACAAAAGTGACCTAGAGCC	ACGTTGGATGTTCTTGCAGGACATTGTGCC
rs1023372	ACGTTGGATGCAAATTCAAAATTCTGTTG	ACGTTGGATGCTCAGAAGTAACATGTACTC
rs2212604	ACGTTGGATGCAGACTTGAGCATATACCAC	ACGTTGGATGACCCATGTGGGAAAATGTTG
rs2226684	ACGTTGGATGGGTGTTGGAAAAGGAACATC	ACGTTGGATGTTAATGATAGTTCCCCTCAG
rs2212605	ACGTTGGATGATATGAGTGATTGTCATGGG	ACGTTGGATGTGCATATAAGCTGTCTGCAC
rs2187307	ACGTTGGATGCACATCCTGCAGCTTTAACC	ACGTTGGATGCCTGGCACTTTCAAGTAACG
rs3065412	ACGTTGGATGGGCTGAGATAGAATGTGCTC	ACGTTGGATGTCTCCTGCTTTGTTCTGGAG
rs2898355	ACGTTGGATGGGCTGAGATAGAATGTGCTC	ACGTTGGATGTCTCCTGCTTTGTTCTGGAG
rs2836518	ACGTTGGATGCACTTGTGCTTCTTCCACC	ACGTTGGATGATGCCAACCTTGCTGATGTC
rs3838110	ACGTTGGATGGAAGTAGTGAAGTGTTCCCC	ACGTTGGATGAGCCTCACTGAATCTTAACG
rs2836519	ACGTTGGATGTGTTTCTCCTTCTCACTGGG	ACGTTGGATGAAAGGCTACAGGAACGAGC
rs3827207	ACGTTGGATGTGTAGTCTGCACCTTCACT	ACGTTGGATGAGCGGCTGCTGAACATAGAT
rs2836520	ACGTTGGATGCCTGCAAAGGTGTTTGCTTC	ACGTTGGATGGCCACCTAATTTTCTCTCTC
rs2836521	ACGTTGGATGAAGAATAAGAAGCAAACACC	ACGTTGGATGGTTTTAGGGGAAAGGCATAAG
rs2836522	ACGTTGGATGTGCATCTTTGGTTGTGACAG	ACGTTGGATGGCACATCTACTCTTAGCATG
rs2836523	ACGTTGGATGTCTCTCTTTCTTTCCCTAC	ACGTTGGATGACTCTCAGTTATGATTTCTC
rs2836524	ACGTTGGATGGTGTGTTGGTAGAAACGTTT	ACGTTGGATGGTCACCCCTTCAGATAATAAG
rs2836525	ACGTTGGATGCAGAGCCGAAAACATAGTTC	ACGTTGGATGGTGTGTTGGTAGAAACGTTT
rs3833350	ACGTTGGATGGTTGTTCTTTTGTCTTCTAG	ACGTTGGATGGAATCATGTCCTTCAGTAAGC
rs2836526	ACGTTGGATGATTGTGCTCCTGCTCCTGCTAG	ACGTTGGATGGACGGCTAGAAGACAAAAGG
rs2836527	ACGTTGGATGGTGTGTTTATGTTCTAGCAGG	ACGTTGGATGGATGCCTTTAGGCAAACATG
rs3834676	ACGTTGGATGAAGCTGAAAAGGATGTGCAG	ACGTTGGATGACAGGGCATACTTCTCTATC
rs2836528	ACGTTGGATGCCAAAACCTCATGCGATCTGC	ACGTTGGATGTGGCGCTGAAGTACTCAATG
rs3761364	ACGTTGGATGAAACAGCACAGCTACCATT	ACGTTGGATGATGAGAAAATGTGTGTGGAG
rs2836529	ACGTTGGATGAGCGGTGTTTTAAATGTCC	ACGTTGGATGCAGAGCCCCAAAAAATTTGG
rs2836530	ACGTTGGATGACAGACAGTGGTCAGAACAT	ACGTTGGATGAAAGATGCCTATAATCCAGG
rs3761366	ACGTTGGATGCAGGTGATAAAAAGCAAGTG	ACGTTGGATGGCCATCAGTTCTTTTTTGGC
rs2836531	ACGTTGGATGGCCTTCGAAAATGTCTCAAG	ACGTTGGATGCACTTGCTTTTTATCACCTG
rs2836532	ACGTTGGATGGAAGACAGCCTTCGAAAATG	ACGTTGGATGCAATGGCTCTTTCAGTAAC
rs2836533	ACGTTGGATGTTTCTGACCTCTCACGGTAC	ACGTTGGATGTGCAGATCTGGAGGTAGATG
rs2836534	ACGTTGGATGAGAAGAGGCTGGGAGAGGAT	ACGTTGGATGTGCTGCTCTTAGGATAAGGG
rs2836535	ACGTTGGATGACAGGAGGAGTTGAGTGTTG	ACGTTGGATGTAGAGGCACGGAGAAGATAG

dbSNP rs#	Forward PCR primer	Reverse PCR primer
rs2836536	ACGTTGGATGAAAAGCATGGGTACAGGAGG	ACGTTGGATGTAGAGGCACGGAGAAGATAG
rs3827208	ACGTTGGATGGAGGATGAGAGGTACCTGAG	ACGTTGGATGGGGATGATCAAACGTAGT
rs715860	ACGTTGGATGTTCTGGTGGAGGTTTCTTGG	ACGTTGGATGCGAGACATGATCTCAAACCC
rs717231	ACGTTGGATGCAAGAGACTCAAACAGTTGC	ACGTTGGATGTCATAGAAGTTACAGCAGCC
rs2836537	ACGTTGGATGTTGGTGTGTGATCACTCTGG	ACGTTGGATGGAACCTAAGTTTCTCCCAGC
rs2836538	ACGTTGGATGGGTTAGAGCTTACGTAATTC	ACGTTGGATGCTACTTGTGTCACTTCTTTG
rs2836539	ACGTTGGATGTTATCCTCCAAGAGCCTTAG	ACGTTGGATGGGGCAAATGGAGTTCTTATT
rs2836540	ACGTTGGATGCCCAGTTGGTATCAGTGTTG	ACGTTGGATGTGCTGAACATCGTTTGGAGG
rs2836541	ACGTTGGATGCTTGCACTGACACCTTTGTG	ACGTTGGATGGTACTGGCGAAGACATGATG
rs2836542	ACGTTGGATGAGATGAGCCATTTCTACTG	ACGTTGGATGCAGCATGAGAACTGAATGC
rs2836543	ACGTTGGATGAAATGGACTTCTTCAGTAGG	ACGTTGGATGGATACAATTCAACCCATAGC
rs881837	ACGTTGGATGAATGGATGTGGCTCTTGAGG	ACGTTGGATGTATGGAGGGACTTACGAAAG
rs3949052	ACGTTGGATGTTTTCAACGAAACAGATGC	ACGTTGGATGCCAAGTAAATATTCAATCCCC
rs2065307	ACGTTGGATGTTTTCAACGAAACAGATGC	ACGTTGGATGCCAAGTAAATATTCAATCCCC
rs3216105	ACGTTGGATGACCACCATGCCTGGCTAATT	ACGTTGGATGGGCCTGGACAAAATAGTGAG
rs2073427	ACGTTGGATGTTTTGCTTGGGTGTTCTGCC	ACGTTGGATGGGATTTACACTGGTGTTGGG

**TABLE 30**

dbSNP rs#	Extend Primer	Term Mix
Rs2898353	TCCTGTCTTCAGTGCTTGATTCTG	CGT
rs960818	AGTAGATAACATAAAGTAACCAGC	ACT
rs960819	GCTATTCACCCTAGCTGTACATAG	ACT
Rs2410034	AAATGTAGCTGTAGTATCTTGAA	ACT
Rs2836437	TTCACACTCAACAACAAACACA	ACT
Rs2836438	TGGAAAGTAAGCTAGACCAAACAG	ACT
Rs2836439	GTATAAAGTGATGCTGCTTGC	ACT
Rs2836440	AACAATTGGGATATGTCTCTCCAC	ACG
Rs2226683	GAGAGTTAATGTGCCCTACTT	ACT
Rs2836441	TAATAGTGCTGGCCATAATGC	ACT
Rs2836442	CTCTAGGCTTACAGTAAACAC	ACT
Rs2836443	TATAAGTTCAGGGTCACAGGTC	ACT
Rs2836444	TGTGTTCTTGGGGTCGCCT	ACT
Rs3787906	TAATGTAGGTGCTGAGAACTTAG	ACT
Rs3838108	GGCTGATTAAAATTCTGTTTCCCC	ACT
Rs2836445	AGACGCAGTAAACTTATGGAT	ACG
Rs2836446	GCCTTGTCCTATCAAGAGCCAAAG	CGT
Rs3787908	CATACAGTAGCTGTGGACAGC	ACT
Rs2836447	ATGTATTACATTGAGAACCATGTG	ACT
Rs2836448	TGTATAGGGCAGGGATAAAGAC	ACT
Rs2836450	AACAACAAATTTACTGATATCATC	ACT
Rs2836451	CTGTCACCCATTGACCTCAC	ACT
Rs1015022	CTTTTATCTGCAGTTGCACCC	ACT
Rs2836452	CGGGAAGATGGCTGCCTTC	ACG

dbSNP rs#	Extend Primer	Term Mix
Rs2836453	CCAAGATGGCCCAGTAGGA	CGT
Rs3787909	AAATAGTAAAATAAAAAAGAGCTCC	ACG
Rs2836454	CACAACCTCCCAAATGAATAAATC	ACT
Rs2836455	TGCTGTGACATTTTAGTGCTTCTG	CGT
Rs2155718	CTCACACACAGCTGGAGTTTA	ACT
Rs2836456	CGTTCTGAAGGTTTTGTGTACA	ACT
Rs2836457	GAGTCACCCGTCCCCTAGA	ACT
Rs2836458	ACAGAAGAGCCAGCCGACA	ACT
Rs2032323	TGCACACTCACTGAAGCCC	ACT
Rs2051400	AAACACTATGTGACGCCACC	ACT
Rs2836459	AGAATGTTACTTTCTGGATTCTAC	ACT
Rs2836460	ATTGTAATTCTCCGTAACCC	ACG
Rs2836461	TACCCACACGGAATTCTCATCTAC	ACT
Rs2836462	TCCGTATGTCTCCATCCATCTCA	ACT
Rs2836463	AAACTTAAATTGCTTTAATCAGCT	ACT
Rs2836464	AATATCTTATCACTGCTCCTGTCT	ACG
Rs2836465	GCCCACTTTTGTGTTTGCTTAG	ACT
Rs2836466	TTTGCCCACTTTTGTGTTTGCT	ACT
Rs2836467	TTAATTTTCTTGCTCTTTCTGTA	ACT
Rs3827204	CCCTCACATCTTCCCCGC	ACT
Rs2836468	GCAGGAAAGAGCATGGGCATTAAC	ACT
Rs3787911	TACATCCAAAAGCCTGCCAG	ACT
Rs2836469	TCCTGCGAGATCCTGCTCA	ACG
Rs2836470	ACAAGCTTAATGTTTTGTTGAGA	ACT
Rs2212599	TTCCCCAACAATAGTCAGAAAA	ACT
Rs2836472	TTCTCTATCATGATGCAGTCC	ACT
Rs2836473	GATGATGAACAGGGCTGTGA	ACG
Rs1888469	AAGGGTCTGAAGAGGAGGC	ACT
Rs1888470	GTTTTCTGCCTCTGATCCTCA	ACT
Rs2032322	CCTATAGGTAACGTGGCTTCT	ACT
Rs2410035	AGGCAGAAGTTGCAGTGAAC	ACG
Rs1573332	GAGAGGCCAGAAAGCCTTC	CGT
Rs2836474	GCACAGGAGAGTCCTCAATT	ACG
Rs2836475	CATGGGAAGCTGCTGAACTA	ACT
Rs3787914	ACAGTGTTTGAGCCCTCCTT	ACT
Rs1888471	AACTGACAGAAGAAAGAAAAATAT	ACG
Rs1888472	TGTGTTGGTGTATAAATCAAGATT	ACG
Rs1888473	CAGTTCTCAGCCAGACGATC	ACG
Rs1888474	GAGTCCAGGGTGCTAATTC	ACG
Rs2836476	GGTGTTAGCCCTGGGTTCTAATAA	ACG
Rs3787916	TCTCTTATGTAAATACAAAGACG	CGT
Rs2836477	CCTCTTAAATAGCCTGCCTTCA	ACT
rs970043	GCTCCTTGACTCAAGTATTC	ACG
Rs2212600	AAAACAACCTTCTCTCCCAAAC	ACG

dbSNP rs#	Extend Primer	Term Mix
Rs2836478	CTTGCTTATCTTCAAGCAGTC	CGT
Rs2836479	CCTAATAGGTGTGAAGTGTA AAA	CGT
Rs1475877	CTCCCCGTGTTCTGCATGC	ACG
Rs2836480	CCCACTGTACATCTTACACTC	ACT
Rs2836481	TCCCCCTGAAATCCCATAGC	ACT
Rs2836483	AGGTAATCTCCAACCAAACCT	ACT
Rs2836484	AGTCATCAAGCCATATCTCCA	ACG
Rs2836485	CTCCTCTGGGACGTCAGC	ACT
Rs2836486	CCTCTAAGTTTAGTGGTGGAT	ACT
Rs2836487	TGTTGGGTTCTACACATTCAAA	ACT
Rs1893199	CAGACCATGGTTTTGAATGTG	ACG
Rs2836488	GTAGAACCCAACACAGAGCC	ACG
Rs1893200	AGTCTTCGCATAACCAAAACAGA	ACT
Rs1893201	CGCATAACCAAAACAGAAAAGAAC	ACT
Rs2836489	CAAGAGCTCTTTTCAATTCCAG	ACT
Rs1888475	GACATCAAATGATTCCCCTGT	ACT
Rs2836490	GAGCCAAAGCTTTCCTGATG	ACT
Rs2836491	GTGGAAAGGGCACTGTGGT	ACT
Rs2836492	GGCATGGAAGAGCAAGCATC	ACT
Rs2836493	TCCGATGCTTGCTCTTCCAT	ACT
Rs2836494	TGAAGTTTCGTTAAATTCAC TACA	ACT
Rs2836495	CTTCAGCAATTCAAATGAATGCAC	ACT
Rs2898354	TCCGGCACATATATCCTGGAAC	ACT
Rs3065390	AAACAAACAAACAAAAACAGTGTA	ACT
Rs2836496	GTGTTCTGATGTTTCTGGAGT	CGT
Rs2836497	CTGCTAGACATTGTCAGTCC	ACT
Rs2836498	AATAGGATGAGTCAAAGAAGGAG	ACT
Rs2836499	GAGAAGAGCCCTGAGTTGATAAA	ACT
Rs2836500	AGAGGATGAGCAATTCAGGGA	ACT
Rs2836501	CAAAGGTTGACCTTGTTTCTAT	CGT
Rs2836502	AAGAACTTACATTTTATGGCTTC	ACT
Rs2836503	GATTTGGGAGCAAGGGAGC	ACT
Rs2836504	AGAGTTAAAGATGACTCTAGGCTC	ACT
Rs3787917	GCAGCCAGAGTGGAGCAGT	ACG
Rs2836505	AAGGCATTCTCCTCCAAATCAC	ACT
Rs2836506	GAAAAATCAAATCAGTTTCTACAAC	ACT
Rs2836507	GTGTTGGAATATTGTTGGCCT	ACT
Rs2836508	ATTCTCTACCATTTTATTCTCTTT	ACT
Rs2836509	TTTCTAAGTCAATGTAGGCAAC	ACT
Rs2836510	CAGCTAGTTATCTTACTTCACC	ACT
Rs2836511	AGCAGGTGACAACCCAGACAT	ACT
Rs2212601	TAAGTTTCTGTTGTTTATATGCCA	ACT
Rs2212602	CCAGCCAGAGATGGGATCA	ACG
Rs2226682	GATTGAACCAGGAAAGAAATAGTT	CGT

dbSNP rs#	Extend Primer	Term Mix
Rs2836512	AATGCCAGTTGCCATAGGATA	ACG
Rs2836513	ATAAGAAGATGAGTACTATTATTG	ACT
Rs1999328	ATTGAGGGAAGAGTAAATGATTTTC	CGT
Rs2212603	TGTCTGTGAGTACCCCAACAATGAA	ACT
Rs3787919	TCTGTGGCTTCAATGCTGGG	ACT
Rs2836514	ACAGACTTTAACAAAATCACTGA	ACT
Rs1023153	GGGTCATCTCCTTACCTGTCCAA	ACG
Rs1023372	TTCCAAAATTCTGGTTGTGTTTT	ACT
Rs2212604	CTGCCCTATACATACATAGCTTC	ACG
Rs2226684	AAAAACAATCTGCACAACAAATAT	ACT
Rs2212605	GCAGTGAATATGAACAAAAAAAAA	ACT
Rs2187307	CAGCTTTAACCTCACTCCAC	ACT
Rs3065412	AGTTACAAATCAGGTGGTGCTGG	ACT
Rs2898355	GTTACAAATCAGGTGGTGCTG	ACT
Rs2836518	TAGGAATCGGAGTCAATAATTTT	ACT
Rs3838110	GCTGCACAATCCCCCCCC	CGT
Rs2836519	CCTTCTCACTGGGTTCTCTG	ACG
Rs3827207	TATCACCCCTGTGTCTCTGC	ACG
Rs2836520	CACAAATAGATTATATATCCTGTT	ACT
Rs2836521	AATAAGAAGCAAACACCTTTGCA	ACT
Rs2836522	CCACCCCTTCAGAGAGTTG	ACT
Rs2836523	TCATATTGGTTGATCGTATTGGTT	ACT
Rs2836524	GATTTCAAGGAATGAACTATGTTTT	ACG
Rs2836525	AGCCGAAAACATAGTTCATTCTCTG	ACT
Rs3833350	CTTTTGTCTTCTAGCCGTCAG	ACT
Rs2836526	AGAACATAAAACACAGAAATGCA	ACT
Rs2836527	TTATGTTCTAGCAGGACAGGA	CGT
Rs3834676	AAAAGGATGTGCAGATCGCAT	ACT
Rs2836528	ATCTGCACATCCTTTTCAGCTT	ACG
Rs3761364	CTACCATTCAATTGAGTACTTCAG	ACG
Rs2836529	CTTCAAAATGTGGGTTGATACC	ACT
Rs2836530	GGTCAGAACATGCTGCTTTAT	ACT
Rs3761366	GTGATGGCTTCTAAAAATGTAAA	ACG
Rs2836531	GCATTTGTTACTGCAAAGAGCCAT	ACG
Rs2836532	AGCCTTCGAAAATGTCTCAAG	CGT
Rs2836533	CACACCCATTCCAACCCAAT	ACG
Rs2836534	GCTGAAGGTTTCTGGGAGCA	ACG
Rs2836535	GAGGAGTTGAGTGTGGAACCA	ACG
Rs2836536	ATGGGTACAGGAGGAGTTGA	ACT
Rs3827208	CACCCACCCCAATCACCC	ACT
rs715860	CTTGGTTATCCTTCAGTTTCCA	ACT
rs717231	CTCATTTAGTTTATGTCTTGGTTG	ACT
Rs2836537	GCTCATACGCCCTTGGTCTCTAAT	ACT
Rs2836538	AGCTTACGTAATTCAAATCAAGT	ACT

dbSNP rs#	Extend Primer	Term Mix
Rs2836539	TTACACATTTGCACAATGAGGATA	CGT
Rs2836540	GTATCAGTGTGAATGACTGGT	ACT
Rs2836541	TGACACCTTTGTGAATTGCTGAAC	ACT
Rs2836542	CCATTTCCTACTGAAGAAGTCCA	ACT
Rs2836543	CTTCTTCAGTAGGAAATGGCT	ACG
rs881837	GGCTCTTGAGGCCATGCC	ACG
Rs3949052	ACAATTTCTCATGTTGTAAGGATT	ACG
Rs2065307	GGAAACAGATGCCATTTACAATTT	ACG
Rs3216105	GCCTGGCTAATTTTTAAAAAAAAA	CGT
Rs2073427	CTGCCCCACATGACCCA	ACG

### Genetic Analysis

[0254] Allelotyping results from the discovery cohort are shown for cases and controls in Table 31. The allele frequency for the A2 allele is noted in the fifth and sixth columns for osteoarthritis case pools and control pools, respectively, where “AF” is allele frequency. The allele frequency for the A1 allele can be easily calculated by subtracting the A2 allele frequency from 1 (A1 AF = 1-A2 AF). For example, the SNP rs2898353 has the following case and control allele frequencies: case A1 (A) = 0.79; case A2 (T) = 0.21; control A1 (A) = 0.81; and control A2 (T) = 0.19, where the nucleotide is provided in paranthesis. Some SNPs are labeled “untyped” because of failed assays.

TABLE 31

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2898353	231	38783681	A/T	0.21	0.19	0.560
rs960818	882	38784332	A/G	0.59	0.57	0.330
rs960819	960	38784410	A/C	0.13	0.09	0.101
rs2410034	1194	38784644	A/C			
rs2836437	1530	38784980	A/G	0.14	0.14	0.956
rs2836438	1673	38785123	A/G	0.79	0.75	0.077
rs2836439	2096	38785546	C/T	0.70	0.71	0.508
rs2836440	2285	38785735	A/G	0.19	0.18	0.623
rs2226683	5873	38789323	C/T	0.79	0.76	0.312
rs2836441	7256	38790706	A/G	0.12	0.12	0.765
rs2836442	7988	38791438	A/G	0.31	0.30	0.746
rs2836443	8222	38791672	G/T	0.22	0.23	0.728
rs2836444	8381	38791831	C/T	0.19	0.20	0.807
rs3787906	8814	38792264	C/T	0.97	untyped	NA
rs3838108	8915	38792365	-/C	0.58	0.56	0.425
rs2836445	9642	38793092	A/G	0.32	0.35	0.190
rs2836446	9902	38793352	A/T	0.12	0.14	0.274
rs3787908	10619	38794069	A/G			
rs2836447	10927	38794377	C/T	0.68	0.67	0.816
rs2836448	11032	38794482	C/T	0.12	0.14	0.235
rs2836450	14377	38797827	C/T	0.70	0.68	0.460
rs2836451	15608	38799058	C/T	0.92	0.95	0.157

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs1015022	15928	38799378	C/G	0.31	0.36	0.072
rs2836452	16296	38799746	A/G	0.18	0.18	0.822
rs2836453	17598	38801048	A/T	0.02	0.02	0.836
rs3787909	19272	38802722	A/G	0.06	0.03	0.091
rs2836454	20084	38803534	A/G	0.04	0.03	0.397
rs2836455	20577	38804027	A/T	0.17	0.13	0.050
rs2155718	28051	38811501	A/G	0.78	0.78	0.950
rs2836456	29466	38812916	A/G	0.94	0.92	0.569
rs2836457	29530	38812980	C/T			
rs2836458	29987	38813437	A/G	0.48	0.46	0.455
rs2032323	30012	38813462	C/T			
rs2051400	30322	38813772	G/T	0.03	NA	NA
rs2836459	32216	38815666	C/T	0.19	0.17	0.319
rs2836460	32516	38815966	C/T			
rs2836461	32544	38815994	A/G			
rs2836462	32746	38816196	A/G			
rs2836463	33137	38816587	G/T	0.67	0.72	0.032
rs2836464	33538	38816988	A/G	0.67	0.67	0.991
rs2836465	33798	38817248	C/T			
rs2836466	33802	38817252	A/C	0.39	0.40	0.627
rs2836467	33964	38817414	C/T			
rs3827204	34132	38817582	A/G	0.45	0.42	0.213
rs2836468	34210	38817660	C/T	0.13	0.14	0.678
rs3787911	34317	38817767	A/G	0.13	0.12	0.862
rs2836469	34499	38817949	C/T	0.38	0.40	0.250
rs2836470	34753	38818203	A/C	0.73	0.74	0.939
rs2212599	34845	38818295	C/T	0.66	0.64	0.474
rs2836472	35335	38818785	C/T	0.40	0.35	0.071
rs2836473	36423	38819873	C/T	0.53	0.54	0.755
rs1888469	36450	38819900	A/G	0.45	0.49	0.175
rs1888470	36481	38819931	G/T	0.17	0.18	0.623
rs2032322	38447	38821897	C/G	0.50	0.50	0.879
rs2410035	38784	38822234	C/T			
rs1573332	39387	38822837	A/T	0.57	0.58	0.609
rs2836474	39458	38822908	C/T	0.33	0.35	0.564
rs2836475	39822	38823272	C/G	0.17	0.14	0.113
rs3787914	40305	38823755	C/G	0.73	0.73	0.987
rs1888471	40869	38824319	C/T	0.29	0.26	0.175
rs1888472	40926	38824376	C/T	0.62	0.63	0.818
rs1888473	41010	38824460	C/T	0.63	0.65	0.435
rs1888474	41134	38824584	C/T	0.28	0.23	0.099
rs2836476	41984	38825434	A/G	0.46	0.44	0.379
rs3787916	42172	38825622	A/T	0.45	0.43	0.314
rs2836477	42753	38826203	G/T	0.94	0.96	0.196
rs970043	43011	38826461	C/T	0.04	0.04	0.549
rs2212600	43176	38826626	A/G			
rs2836478	43320	38826770	G/T	0.76	0.75	0.914
rs2836479	43381	38826831	A/T	0.44	0.43	0.670
rs1475877	44142	38827592	A/G	0.35	0.32	0.110
rs2836480	44383	38827833	A/G	0.46	0.43	0.153
rs2836481	44726	38828176	C/T	0.42	0.40	0.434
rs2836483	45087	38828537	A/G	0.47	0.45	0.393
rs2836484	45141	38828591	C/T	0.46	0.47	0.671
rs2836485	45359	38828809	C/G	0.16	0.17	0.643
rs2836486	45421	38828871	C/T			
rs2836487	45456	38828906	C/T	0.02	0.03	0.758
rs1893199	45467	38828917	C/T	0.62	0.65	0.220

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2836488	45486	38828936	C/T	0.25	0.23	0.360
rs1893200	45709	38829159	A/G	0.16	0.14	0.177
rs1893201	45716	38829166	A/G	0.84	0.87	0.060
rs2836489	47626	38831076	C/T	0.29	0.31	0.502
rs1888475	49413	38832863	A/G			
rs2836490	49796	38833246	C/T	0.94	0.93	0.731
rs2836491	49962	38833412	A/G	0.10	0.08	0.219
rs2836492	50075	38833525	C/T	0.20	0.22	0.518
rs2836493	50093	38833543	A/G	0.95	0.94	0.850
rs2836494	50571	38834021	C/T	0.72	0.70	0.536
rs2836495	50615	38834065	A/G	0.82	0.78	0.142
rs2898354	50780	38834230	A/G	0.25	0.25	0.728
rs3065390	50851	38834301	-T/A	0.10	0.11	0.845
rs2836496	51459	38834909	A/C	0.80	0.84	0.064
rs2836497	53193	38836643	C/T	0.65	0.65	0.935
rs2836498	53702	38837152	C/T	0.43	0.44	0.682
rs2836499	53736	38837186	A/C	0.33	0.30	0.169
rs2836500	53795	38837245	C/T			
rs2836501	54109	38837559	A/T	0.36	0.34	0.234
rs2836502	54126	38837576	C/T	0.31	0.29	0.427
rs2836503	54230	38837680	A/C	0.32	0.29	0.194
rs2836504	54894	38838344	C/T	0.51	0.54	0.170
rs3787917	55455	38838905	A/G	0.56	0.60	0.137
rs2836505	55499	38838949	A/G	0.73	0.78	0.022
rs2836506	56522	38839972	C/T	0.52	0.56	0.145
rs2836507	56662	38840112	C/T	0.51	0.54	0.173
rs2836508	56954	38840404	A/G	0.53	0.56	0.376
rs2836509	57267	38840717	A/G	0.35	0.31	0.089
rs2836510	58282	38841732	A/G	0.65	0.59	0.034
rs2836511	58916	38842366	A/C	0.32	0.30	0.315
rs2212601	59544	38842994	C/G	0.45	0.46	0.568
rs2212602	59666	38843116	C/T	0.30	0.28	0.644
rs2226682	59913	38843363	A/T	0.38	0.35	0.164
rs2836512	66846	38850296	A/G	0.94	0.94	0.896
rs2836513	67245	38850695	G/T	0.23	0.22	0.713
rs1999328	67652	38851102	A/C	0.79	0.79	0.973
rs2212603	67955	38851405	A/G	0.73	0.72	0.776
rs3787919	67966	38851416	A/C			
rs2836514	68420	38851870	A/G	0.52	0.54	0.319
rs1023153	70226	38853676	A/G	0.09	0.09	0.985
rs1023372	70810	38854260	C/T	0.83	0.81	0.518
rs2212604	72246	38855696	A/G	0.68	0.71	0.237
rs2226684	73330	38856780	G/T	0.83	0.81	0.462
rs2212605	73457	38856907	C/T	0.82	0.85	0.255
rs2187307	74389	38857839	A/G	0.13	0.13	0.869
rs3065412	74638	38858088	-A/A			
rs2898355	74640	38858090	A/C	0.96	0.94	0.413
rs2836518	75358	38858808	A/C	0.10	0.12	0.261
rs3838110	75952	38859402	-G	0.66	0.67	0.790
rs2836519	76098	38859548	A/G	0.60	0.61	0.509
rs3827207	77836	38861286	A/G	0.62	0.63	0.575
rs2836520	78449	38861899	A/C			
rs2836521	78507	38861957	G/T	0.07	0.08	0.551
rs2836522	80031	38863481	G/T	0.11	0.08	0.155
rs2836523	81695	38865145	C/T			
rs2836524	82775	38866225	A/G	0.05	0.04	0.321
rs2836525	82795	38866245	A/G	0.11	0.11	0.875

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3833350	84611	38868061	-/C			
rs2836526	84657	38868107	C/T	0.83	0.86	0.292
rs2836527	84693	38868143	A/C	0.08	0.08	0.936
rs3834676	85020	38868470	-/T	0.80	0.83	0.191
rs2836528	85048	38868498	C/T	0.84	0.87	0.089
rs3761364	85100	38868550	C/T	0.06	0.04	0.159
rs2836529	85325	38868775	A/C	0.09	0.06	0.100
rs2836530	85452	38868902	C/T			
rs3761366	85868	38869318	A/G	0.06	0.04	0.179
rs2836531	85936	38869386	A/G	0.49	0.50	0.729
rs2836532	85990	38869440	A/T	0.30	0.29	0.766
rs2836533	86139	38869589	C/T	0.47	0.48	0.751
rs2836534	86497	38869947	C/T	0.87	0.87	0.874
rs2836535	87236	38870686	A/G	0.93	0.92	0.628
rs2836536	87248	38870698	C/T	0.86	0.84	0.474
rs3827208	87533	38870983	C/G	0.51	0.53	0.459
rs715860	87912	38871362	A/G	0.08	0.09	0.627
rs717231	88108	38871558	G/T	0.65	0.67	0.382
rs2836537	88494	38871944	A/C	0.43	0.40	0.239
rs2836538	89598	38873048	A/C			
rs2836539	90235	38873685	A/T	0.98	0.97	0.796
rs2836540	91287	38874737	G/T			
rs2836541	91359	38874809	C/T	0.07	0.06	0.403
rs2836542	92384	38875834	A/C	0.36	0.38	0.418
rs2836543	92410	38875860	C/T	0.54	0.50	0.202
rs881837	92900	38876350	C/T	0.29	0.28	0.639
rs3949052	94495	38877945	A/G			
rs2065307	94512	38877962	A/G			
rs3216105	97777	38881227	-/A	0.32	0.28	0.265
rs2073427	98333	38881783	C/T	0.09	0.07	0.242

[0255] The *ERG* proximal SNPs were also allelotyped in the replication cohorts using the methods described herein and the primers provided in Tables 29 and 30. The replication allelotyping results for replication cohort #1 and replication cohort #2 are provided in Tables 32 and 33, respectively.

TABLE 32

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2898353	231	38783681	A/T	0.19	0.19	0.773
rs960818	882	38784332	A/G	0.59	0.57	0.600
rs960819	960	38784410	A/C	0.07	NA	0.132
rs2410034	1194	38784644	A/C			
rs2836437	1530	38784980	A/G	0.14	0.14	0.957
rs2836438	1673	38785123	A/G	0.80	0.77	0.402
rs2836439	2096	38785546	C/T	0.68	0.73	0.089
rs2836440	2285	38785735	A/G	0.20	0.18	0.421
rs2226683	5873	38789323	C/T	0.78	0.76	0.622
rs2836441	7256	38790706	A/G	0.12	0.12	0.946
rs2836442	7988	38791438	A/G	0.30	0.32	0.674
rs2836443	8222	38791672	G/T	0.22	0.25	0.332
rs2836444	8381	38791831	C/T	0.20	0.20	0.908
rs3787906	8814	38792264	C/T	0.97	untyped	NA

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs3838108	8915	38792365	-/C	0.58	0.56	0.604
rs2836445	9642	38793092	A/G	0.33	0.37	0.211
rs2836446	9902	38793352	A/T	0.13	0.15	0.481
rs3787908	10619	38794069	A/G			
rs2836447	10927	38794377	C/T	0.67	0.67	0.843
rs2836448	11032	38794482	C/T	0.13	0.15	0.521
rs2836450	14377	38797827	C/T	0.67	0.67	0.989
rs2836451	15608	38799058	C/T	0.92	0.95	0.214
rs1015022	15928	38799378	C/G	0.30	0.36	0.076
rs2836452	16296	38799746	A/G	0.18	0.18	0.982
rs2836453	17598	38801048	A/T	0.02	untyped	NA
rs3787909	19272	38802722	A/G	0.06	0.03	0.110
rs2836454	20084	38803534	A/G	0.03	0.03	0.746
rs2836455	20577	38804027	A/T	0.17	0.12	0.080
rs2155718	28051	38811501	A/G	0.78	0.79	0.747
rs2836456	29466	38812916	A/G	0.91	0.91	0.915
rs2836457	29530	38812980	C/T			
rs2836458	29987	38813437	A/G	0.48	0.47	0.626
rs2032323	30012	38813462	C/T			
rs2051400	30322	38813772	G/T	0.02	untyped	NA
rs2836459	32216	38815666	C/T	0.20	0.16	0.278
rs2836460	32516	38815966	C/T			
rs2836461	32544	38815994	A/G			
rs2836462	32746	38816196	A/G			
rs2836463	33137	38816587	G/T	0.67	0.75	0.011
rs2836464	33538	38816988	A/G	0.66	0.68	0.586
rs2836465	33798	38817248	C/T			
rs2836466	33802	38817252	A/C	0.39	0.41	0.507
rs2836467	33964	38817414	C/T			
rs3827204	34132	38817582	A/G	0.45	0.41	0.229
rs2836468	34210	38817660	C/T	0.13	0.14	0.736
rs3787911	34317	38817767	A/G	0.14	0.13	0.856
rs2836469	34499	38817949	C/T	0.37	0.41	0.168
rs2836470	34753	38818203	A/C	0.72	0.73	0.854
rs2212599	34845	38818295	C/T	0.63	0.65	0.636
rs2836472	35335	38818785	C/T	0.41	0.35	0.145
rs2836473	36423	38819873	C/T	0.51	0.54	0.291
rs1888469	36450	38819900	A/G	0.45	0.49	0.281
rs1888470	36481	38819931	G/T	0.17	0.17	0.949
rs2032322	38447	38821897	C/G	0.51	0.53	0.476
rs2410035	38784	38822234	C/T			
rs1573332	39387	38822837	A/T	0.56	0.60	0.279
rs2836474	39458	38822908	C/T	0.33	0.36	0.330
rs2836475	39822	38823272	C/G	0.18	0.13	0.049
rs3787914	40305	38823755	C/G	0.73	0.74	0.977
rs1888471	40869	38824319	C/T	0.31	0.26	0.134
rs1888472	40926	38824376	C/T	0.62	0.65	0.247
rs1888473	41010	38824460	C/T	0.63	0.67	0.210
rs1888474	41134	38824584	C/T	0.28	0.21	0.091
rs2836476	41984	38825434	A/G	0.47	0.44	0.346
rs3787916	42172	38825622	A/T	0.46	0.41	0.171
rs2836477	42753	38826203	G/T	0.94	0.97	0.294
rs970043	43011	38826461	C/T	0.05	0.03	0.331
rs2212600	43176	38826626	A/G			
rs2836478	43320	38826770	G/T	0.75	0.75	0.983
rs2836479	43381	38826831	A/T	0.44	0.43	0.752
rs1475877	44142	38827592	A/G	0.35	0.31	0.166

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2836480	44383	38827833	A/G	0.45	0.41	0.254
rs2836481	44726	38828176	C/T	0.42	0.39	0.330
rs2836483	45087	38828537	A/G	0.46	0.46	0.797
rs2836484	45141	38828591	C/T	0.45	0.47	0.553
rs2836485	45359	38828809	C/G	0.18	0.18	0.993
rs2836486	45421	38828871	C/T			
rs2836487	45456	38828906	C/T	0.03	0.03	0.955
rs1893199	45467	38828917	C/T	0.61	0.67	0.071
rs2836488	45486	38828936	C/T	0.27	0.23	0.246
rs1893200	45709	38829159	A/G	0.16	0.13	0.203
rs1893201	45716	38829166	A/G	0.83	0.89	0.021
rs2836489	47626	38831076	C/T	0.30	0.31	0.702
rs1888475	49413	38832863	A/G			
rs2836490	49796	38833246	C/T	0.94	0.95	0.662
rs2836491	49962	38833412	A/G	0.10	0.06	0.038
rs2836492	50075	38833525	C/T	0.20	0.22	0.651
rs2836493	50093	38833543	A/G	0.93	0.95	0.397
rs2836494	50571	38834021	C/T	0.73	0.71	0.592
rs2836495	50615	38834065	A/G	0.81	0.77	0.212
rs2898354	50780	38834230	A/G	0.24	0.24	0.827
rs3065390	50851	38834301	-/TA	0.10	0.11	0.743
rs2836496	51459	38834909	A/C	0.78	0.86	0.022
rs2836497	53193	38836643	C/T	0.65	0.66	0.733
rs2836498	53702	38837152	C/T	0.44	0.46	0.576
rs2836499	53736	38837186	A/C	0.33	0.29	0.200
rs2836500	53795	38837245	C/T			
rs2836501	54109	38837559	A/T	0.36	0.32	0.167
rs2836502	54126	38837576	C/T	0.31	0.27	0.206
rs2836503	54230	38837680	A/C	0.32	0.28	0.173
rs2836504	54894	38838344	C/T	0.50	0.57	0.033
rs3787917	55455	38838905	A/G	0.56	0.62	0.033
rs2836505	55499	38838949	A/G	0.72	0.81	0.004
rs2836506	56522	38839972	C/T	0.52	0.58	0.093
rs2836507	56662	38840112	C/T	0.51	0.56	0.134
rs2836508	56954	38840404	A/G	0.53	0.58	0.170
rs2836509	57267	38840717	A/G	0.35	0.30	0.136
rs2836510	58282	38841732	A/G	0.62	0.56	0.035
rs2836511	58916	38842366	A/C	0.33	0.30	0.273
rs2212601	59544	38842994	C/G	0.44	0.46	0.675
rs2212602	59666	38843116	C/T	0.29	0.27	0.571
rs2226682	59913	38843363	A/T	0.38	0.33	0.127
rs2836512	66846	38850296	A/G	0.93	0.96	0.261
rs2836513	67245	38850695	G/T	0.23	0.22	0.692
rs1999328	67652	38851102	A/C	0.79	0.80	0.618
rs2212603	67955	38851405	A/G	0.73	0.74	0.676
rs3787919	67966	38851416	A/C			
rs2836514	68420	38851870	A/G	0.51	0.57	0.044
rs1023153	70226	38853676	A/G	0.09	0.09	0.699
rs1023372	70810	38854260	C/T	0.82	untyped	NA
rs2212604	72246	38855696	A/G	0.67	0.73	0.063
rs2226684	73330	38856780	G/T	0.82	0.82	0.992
rs2212605	73457	38856907	C/T	0.83	0.86	0.180
rs2187307	74389	38857839	A/G	0.14	0.13	0.901
rs3065412	74638	38858088	-/AA			
rs2898355	74640	38858090	A/C	0.95	0.93	0.442
rs2836518	75358	38858808	A/C	0.11	0.14	0.248
rs3838110	75952	38859402	-/G	0.65	0.68	0.399

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2836519	76098	38859548	A/G	0.59	0.64	0.134
rs3827207	77836	38861286	A/G	0.60	0.64	0.205
rs2836520	78449	38861899	A/C			
rs2836521	78507	38861957	G/T	0.08	0.09	0.765
rs2836522	80031	38863481	G/T	0.12	0.07	0.033
rs2836523	81695	38865145	C/T			
rs2836524	82775	38866225	A/G	0.05	0.04	0.539
rs2836525	82795	38866245	A/G	0.12	0.09	0.179
rs3833350	84611	38868061	-/C			
rs2836526	84657	38868107	C/T	0.83	0.85	0.536
rs2836527	84693	38868143	A/C	0.08	0.07	0.444
rs3834676	85020	38868470	-/T	0.79	0.82	0.270
rs2836528	85048	38868498	C/T	0.82	0.86	0.130
rs3761364	85100	38868550	C/T	0.08	0.05	0.132
rs2836529	85325	38868775	A/C	0.09	0.07	0.214
rs2836530	85452	38868902	C/T			
rs3761366	85868	38869318	A/G	0.07	0.04	0.259
rs2836531	85936	38869386	A/G	0.49	0.50	0.741
rs2836532	85990	38869440	A/T	0.30	0.30	0.921
rs2836533	86139	38869589	C/T	0.48	0.48	0.843
rs2836534	86497	38869947	C/T	0.86	0.89	0.374
rs2836535	87236	38870686	A/G	0.91	0.91	0.933
rs2836536	87248	38870698	C/T	0.86	0.86	0.945
rs3827208	87533	38870983	C/G	0.51	0.55	0.183
rs715860	87912	38871362	A/G	0.07	0.07	0.893
rs717231	88108	38871558	G/T	0.65	0.68	0.506
rs2836537	88494	38871944	A/C	0.43	0.39	0.251
rs2836538	89598	38873048	A/C			
rs2836539	90235	38873685	A/T	0.98	0.98	0.910
rs2836540	91287	38874737	G/T			
rs2836541	91359	38874809	C/T	0.09	0.06	0.324
rs2836542	92384	38875834	A/C	0.37	0.41	0.365
rs2836543	92410	38875860	C/T	0.54	0.55	0.863
rs881837	92900	38876350	C/T	0.30	0.28	0.673
rs3949052	94495	38877945	A/G			
rs2065307	94512	38877962	A/G			
rs3216105	97777	38881227	-/A	0.31	0.29	0.603
rs2073427	98333	38881783	C/T	0.09	0.06	0.249

TABLE 33

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2898353	231	38783681	A/T	0.22	0.21	0.629
rs960818	882	38784332	A/G	0.59	0.55	0.351
rs960819	960	38784410	A/C	0.12	0.01	
rs2410034	1194	38784644	A/C			
rs2836437	1530	38784980	A/G	0.14	0.14	0.989
rs2836438	1673	38785123	A/G	0.78	0.71	0.047
rs2836439	2096	38785546	C/T	0.72	0.68	0.265
rs2836440	2285	38785735	A/G	0.18	0.19	0.789
rs2226683	5873	38789323	C/T	0.80	0.77	0.342
rs2836441	7256	38790706	A/G	0.11	0.12	0.559
rs2836442	7988	38791438	A/G	0.32	0.28	0.269
rs2836443	8222	38791672	G/T	0.23	0.21	0.504

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2836444	8381	38791831	C/T	0.19	0.19	0.829
rs3787906	8814	38792264	C/T	0.97	untyped	
rs3838108	8915	38792365	-/C	0.58	0.55	0.526
rs2836445	9642	38793092	A/G	0.30	0.32	0.722
rs2836446	9902	38793352	A/T	0.11	0.14	0.425
rs3787908	10619	38794069	A/G			
rs2836447	10927	38794377	C/T	0.68	0.68	0.908
rs2836448	11032	38794482	C/T	0.11	0.14	0.302
rs2836450	14377	38797827	C/T	0.73	0.70	0.314
rs2836451	15608	38799058	C/T	0.93	0.94	0.499
rs1015022	15928	38799378	C/G	0.33	0.35	0.527
rs2836452	16296	38799746	A/G	0.17	0.18	0.750
rs2836453	17598	38801048	A/T	0.02	0.02	0.934
rs3787909	19272	38802722	A/G	0.05	0.04	0.546
rs2836454	20084	38803534	A/G	0.05	0.03	0.379
rs2836455	20577	38804027	A/T	0.17	0.15	0.472
rs2155718	28051	38811501	A/G	0.79	0.78	0.704
rs2836456	29466	38812916	A/G	0.97	0.94	0.174
rs2836457	29530	38812980	C/T			
rs2836458	29987	38813437	A/G	0.48	0.45	0.532
rs2032323	30012	38813462	C/T			
rs2051400	30322	38813772	G/T	0.04	0.02	0.476
rs2836459	32216	38815666	C/T	0.19	0.18	0.921
rs2836460	32516	38815966	C/T			
rs2836461	32544	38815994	A/G			
rs2836462	32746	38816196	A/G			
rs2836463	33137	38816587	G/T	0.68	0.68	0.988
rs2836464	33538	38816988	A/G	0.69	0.66	0.430
rs2836465	33798	38817248	C/T			
rs2836466	33802	38817252	A/C	0.39	0.39	0.948
rs2836467	33964	38817414	C/T			
rs3827204	34132	38817582	A/G	0.45	0.43	0.614
rs2836468	34210	38817660	C/T	0.12	0.12	0.879
rs3787911	34317	38817767	A/G	0.12	0.11	0.901
rs2836469	34499	38817949	C/T	0.38	0.39	0.914
rs2836470	34753	38818203	A/C	0.75	0.74	0.960
rs2212599	34845	38818295	C/T	0.71	0.64	0.095
rs2836472	35335	38818785	C/T	0.40	0.36	0.321
rs2836473	36423	38819873	C/T	0.56	0.53	0.433
rs1888469	36450	38819900	A/G	0.45	0.49	0.399
rs1888470	36481	38819931	G/T	0.16	0.19	0.356
rs2032322	38447	38821897	C/G	0.50	0.45	0.190
rs2410035	38784	38822234	C/T			
rs1573332	39387	38822837	A/T	0.58	0.56	0.554
rs2836474	39458	38822908	C/T	0.34	0.33	0.762
rs2836475	39822	38823272	C/G	0.15	0.14	0.817
rs3787914	40305	38823755	C/G	0.73	0.73	0.934
rs1888471	40869	38824319	C/T	0.28	0.27	0.760
rs1888472	40926	38824376	C/T	0.63	0.58	0.302
rs1888473	41010	38824460	C/T	0.63	0.62	0.683
rs1888474	41134	38824584	C/T	0.27	0.26	0.853
rs2836476	41984	38825434	A/G	0.46	0.45	0.838
rs3787916	42172	38825622	A/T	0.44	0.45	0.827
rs2836477	42753	38826203	G/T	0.94	0.95	0.505
rs970043	43011	38826461	C/T	0.04	0.04	0.848
rs2212600	43176	38826626	A/G			
rs2836478	43320	38826770	G/T	0.76	0.75	0.893

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2836479	43381	38826831	A/T	0.44	0.43	0.801
rs1475877	44142	38827592	A/G	0.35	0.33	0.450
rs2836480	44383	38827833	A/G	0.47	0.44	0.444
rs2836481	44726	38828176	C/T	0.41	0.41	0.999
rs2836483	45087	38828537	A/G	0.48	0.44	0.306
rs2836484	45141	38828591	C/T	0.46	0.46	0.939
rs2836485	45359	38828809	C/G	0.15	0.17	0.483
rs2836486	45421	38828871	C/T			
rs2836487	45456	38828906	C/T	NA	0.03	NA
rs1893199	45467	38828917	C/T	0.63	0.62	0.868
rs2836488	45486	38828936	C/T	0.23	0.22	0.913
rs1893200	45709	38829159	A/G	0.17	0.16	0.653
rs1893201	45716	38829166	A/G	0.85	0.85	0.947
rs2836489	47626	38831076	C/T	0.27	0.30	0.597
rs1888475	49413	38832863	A/G			
rs2836490	49796	38833246	C/T	0.94	0.91	0.196
rs2836491	49962	38833412	A/G	0.09	0.11	0.493
rs2836492	50075	38833525	C/T	0.20	0.21	0.669
rs2836493	50093	38833543	A/G	0.96	0.93	0.211
rs2836494	50571	38834021	C/T	0.70	0.69	0.697
rs2836495	50615	38834065	A/G	0.82	0.80	0.510
rs2898354	50780	38834230	A/G	0.27	0.26	0.846
rs3065390	50851	38834301	-/TA	0.11	0.10	0.936
rs2836496	51459	38834909	A/C	0.81	0.80	0.746
rs2836497	53193	38836643	C/T	0.66	0.64	0.756
rs2836498	53702	38837152	C/T	0.41	0.40	0.844
rs2836499	53736	38837186	A/C	0.32	0.30	0.567
rs2836500	53795	38837245	C/T			
rs2836501	54109	38837559	A/T	0.36	0.36	0.917
rs2836502	54126	38837576	C/T	0.31	0.32	0.738
rs2836503	54230	38837680	A/C	0.32	0.31	0.730
rs2836504	54894	38838344	C/T	0.52	0.50	0.620
rs3787917	55455	38838905	A/G	0.57	0.56	0.759
rs2836505	55499	38838949	A/G	0.74	0.74	0.982
rs2836506	56522	38839972	C/T	0.52	0.53	0.907
rs2836507	56662	38840112	C/T	0.51	0.52	0.785
rs2836508	56954	38840404	A/G	0.53	0.52	0.709
rs2836509	57267	38840717	A/G	0.35	0.33	0.453
rs2836510	58282	38841732	A/G	0.68	0.65	0.457
rs2836511	58916	38842366	A/C	0.32	0.31	0.832
rs2212601	59544	38842994	C/G	0.45	0.47	0.717
rs2212602	59666	38843116	C/T	0.30	0.30	0.994
rs2226682	59913	38843363	A/T	0.39	0.38	0.801
rs2836512	66846	38850296	A/G	0.94	0.91	0.184
rs2836513	67245	38850695	G/T	0.23	0.23	0.949
rs1999328	67652	38851102	A/C	0.80	0.77	0.487
rs2212603	67955	38851405	A/G	0.74	0.70	0.289
rs3787919	67966	38851416	A/C			
rs2836514	68420	38851870	A/G	0.53	0.49	0.363
rs1023153	70226	38853676	A/G	0.08	0.09	0.611
rs1023372	70810	38854260	C/T	0.84	0.81	0.315
rs2212604	72246	38855696	A/G	0.69	0.68	0.641
rs2226684	73330	38856780	G/T	0.85	0.81	0.216
rs2212605	73457	38856907	C/T	0.82	0.82	0.927
rs2187307	74389	38857839	A/G	0.12	0.13	0.685
rs3065412	74638	38858088	-/AA			
rs2898355	74640	38858090	A/C	0.96	0.96	0.893

dbSNP rs#	Position in SEQ ID NO: 4	Chromosome Position	A1/A2 Allele	F A2 Case AF	F A2 Control AF	F p- Value
rs2836518	75358	38858808	A/C	0.10	0.11	0.823
rs3838110	75952	38859402	-/G	0.68	0.65	0.457
rs2836519	76098	38859548	A/G	0.60	0.57	0.357
rs3827207	77836	38861286	A/G	0.64	0.61	0.449
rs2836520	78449	38861899	A/C			
rs2836521	78507	38861957	G/T	0.06	0.07	0.625
rs2836522	80031	38863481	G/T	0.09	0.10	0.810
rs2836523	81695	38865145	C/T			
rs2836524	82775	38866225	A/G	0.05	0.04	0.419
rs2836525	82795	38866245	A/G	0.10	0.14	0.132
rs3833350	84611	38868061	-/C			
rs2836526	84657	38868107	C/T	0.83	0.86	0.342
rs2836527	84693	38868143	A/C	0.08	0.11	0.209
rs3834676	85020	38868470	-/T	0.81	0.84	0.442
rs2836528	85048	38868498	C/T	0.86	0.88	0.350
rs3761364	85100	38868550	C/T	0.04	0.03	0.643
rs2836529	85325	38868775	A/C	0.08	0.06	0.271
rs2836530	85452	38868902	C/T			
rs3761366	85868	38869318	A/G	0.06	0.04	0.473
rs2836531	85936	38869386	A/G	0.49	0.49	0.915
rs2836532	85990	38869440	A/T	0.31	0.28	0.446
rs2836533	86139	38869589	C/T	0.47	0.48	0.810
rs2836534	86497	38869947	C/T	0.88	0.84	0.149
rs2836535	87236	38870686	A/G	0.94	0.92	0.378
rs2836536	87248	38870698	C/T	0.86	0.82	0.311
rs3827208	87533	38870983	C/G	0.51	0.49	0.598
rs715860	87912	38871362	A/G	0.09	0.11	0.463
rs717231	88108	38871558	G/T	0.65	0.67	0.588
rs2836537	88494	38871944	A/C	0.42	0.41	0.694
rs2836538	89598	38873048	A/C			
rs2836539	90235	38873685	A/T	0.97	0.97	0.749
rs2836540	91287	38874737	G/T			
rs2836541	91359	38874809	C/T	0.05	0.05	0.895
rs2836542	92384	38875834	A/C	0.34	0.34	0.998
rs2836543	92410	38875860	C/T	untyped	0.43	NA
rs881837	92900	38876350	C/T	0.29	0.28	0.811
rs3949052	94495	38877945	A/G			
rs2065307	94512	38877962	A/G			
rs3216105	97777	38881227	-/A	0.32	0.28	0.273
rs2073427	98333	38881783	C/T	0.08	0.07	0.700

[0256] Allelotyping results were considered particularly significant with a calculated p-value of less than or equal to 0.05 for allelotype results. These values are indicated in bold. The allelotyping p-values were plotted in Figure 1D for the discovery cohort. The position of each SNP on the chromosome is presented on the x-axis. The y-axis gives the negative logarithm (base 10) of the p-value comparing the estimated allele in the case group to that of the control group. The minor allele frequency of the control group for each SNP designated by an X or other symbol on the graphs in Figure 1D can be determined by consulting Table 31. For example, the left-most X on the left graph is at position 38783681. By proceeding down the Table from top to bottom and across the graphs from left to right the allele frequency associated with each symbol shown can be determined.

[0257] To aid the interpretation, multiple lines have been added to the graph. The broken horizontal lines are drawn at two common significance levels, 0.05 and 0.01. The vertical broken lines are drawn every 20kb to assist in the interpretation of distances between SNPs. Two other lines are drawn to expose linear trends in the association of SNPs to the disease. The light gray line (or generally bottom-most curve) is a nonlinear smoother through the data points on the graph using a local polynomial regression method (W.S. Cleveland, E. Grosse and W.M. Shyu (1992) Local regression models. Chapter 8 of Statistical Models in S eds J.M. Chambers and T.J. Hastie, Wadsworth & Brooks/Cole.). The black line provides a local test for excess statistical significance to identify regions of association. This was created by use of a 10kb sliding window with 1kb step sizes. Within each window, a chi-square goodness of fit test was applied to compare the proportion of SNPs that were significant at a test wise level of 0.01, to the proportion that would be expected by chance alone (0.05 for the methods used here). Resulting p-values that were less than  $10^{-8}$  were truncated at that value.

[0258] Finally, the exons and introns of the genes in the covered region are plotted below each graph at the appropriate chromosomal positions. The gene boundary is indicated by the broken horizontal line. The exon positions are shown as thick, unbroken bars. An arrow is placed at the 3' end of each gene to show the direction of transcription.

### Example 8

#### *In Vitro* Production of Target Polypeptides

[0259] cDNA is cloned into a pIVEX 2.3-MCS vector (Roche Biochem) using a directional cloning method. A cDNA insert is prepared using PCR with forward and reverse primers having 5' restriction site tags (in frame) and 5-6 additional nucleotides in addition to 3' gene-specific portions, the latter of which is typically about twenty to about twenty-five base pairs in length. A Sal I restriction site is introduced by the forward primer and a Sma I restriction site is introduced by the reverse primer. The ends of PCR products are cut with the corresponding restriction enzymes (*i.e.*, Sal I and Sma I) and the products are gel-purified. The pIVEX 2.3-MCS vector is linearized using the same restriction enzymes, and the fragment with the correct sized fragment is isolated by gel-purification. Purified PCR product is ligated into the linearized pIVEX 2.3-MCS vector and *E. coli* cells transformed for plasmid amplification. The newly constructed expression vector is verified by restriction mapping and used for protein production.

[0260] *E. coli* lysate is reconstituted with 0.25 ml of Reconstitution Buffer, the Reaction Mix is reconstituted with 0.8 ml of Reconstitution Buffer; the Feeding Mix is reconstituted with 10.5 ml of Reconstitution Buffer; and the Energy Mix is reconstituted with 0.6 ml of Reconstitution Buffer. 0.5 ml of the Energy Mix was added to the Feeding Mix to obtain the Feeding Solution. 0.75 ml of Reaction Mix, 50 µl of Energy Mix, and 10 µg of the template DNA is added to the *E. coli* lysate.

**[0261]** Using the reaction device (Roche Biochem), 1 ml of the Reaction Solution is loaded into the reaction compartment. The reaction device is turned upside-down and 10 ml of the Feeding Solution is loaded into the feeding compartment. All lids are closed and the reaction device is loaded into the RTS500 instrument. The instrument is run at 30°C for 24 hours with a stir bar speed of 150 rpm. The pIVEX 2.3 MCS vector includes a nucleotide sequence that encodes six consecutive histidine amino acids on the C-terminal end of the target polypeptide for the purpose of protein purification. Target polypeptide is purified by contacting the contents of reaction device with resin modified with Ni<sup>2+</sup> ions. Target polypeptide is eluted from the resin with a solution containing free Ni<sup>2+</sup> ions.

#### Example 9

##### Cellular Production of Target Polypeptides

**[0262]** Nucleic acids are cloned into DNA plasmids having phage recombination sites and target polypeptides are expressed therefrom in a variety of host cells. Alpha phage genomic DNA contains short sequences known as attP sites, and *E. coli* genomic DNA contains unique, short sequences known as attB sites. These regions share homology, allowing for integration of phage DNA into *E. coli* via directional, site-specific recombination using the phage protein Int and the *E. coli* protein IHF. Integration produces two new att sites, L and R, which flank the inserted prophage DNA. Phage excision from *E. coli* genomic DNA can also be accomplished using these two proteins with the addition of a second phage protein, Xis. DNA vectors have been produced where the integration/excision process is modified to allow for the directional integration or excision of a target DNA fragment into a backbone vector in a rapid *in vitro* reaction (Gateway™ Technology (Invitrogen, Inc.)).

**[0263]** A first step is to transfer the nucleic acid insert into a shuttle vector that contains attL sites surrounding the negative selection gene, ccdB (*e.g.* pENTER vector, Invitrogen, Inc.). This transfer process is accomplished by digesting the nucleic acid from a DNA vector used for sequencing, and to ligate it into the multicloning site of the shuttle vector, which will place it between the two attL sites while removing the negative selection gene ccdB. A second method is to amplify the nucleic acid by the polymerase chain reaction (PCR) with primers containing attB sites. The amplified fragment then is integrated into the shuttle vector using Int and IHF. A third method is to utilize a topoisomerase-mediated process, in which the nucleic acid is amplified via PCR using gene-specific primers with the 5' upstream primer containing an additional CACC sequence (*e.g.*, TOPO® expression kit (Invitrogen, Inc.)). In conjunction with Topoisomerase I, the PCR amplified fragment can be cloned into the shuttle vector via the attL sites in the correct orientation.

**[0264]** Once the nucleic acid is transferred into the shuttle vector, it can be cloned into an expression vector having attR sites. Several vectors containing attR sites for expression of target polypeptide as a

native polypeptide, N-fusion polypeptide, and C-fusion polypeptides are commercially available (*e.g.*, pDEST (Invitrogen, Inc.)), and any vector can be converted into an expression vector for receiving a nucleic acid from the shuttle vector by introducing an insert having an attR site flanked by an antibiotic resistant gene for selection using the standard methods described above. Transfer of the nucleic acid from the shuttle vector is accomplished by directional recombination using Int, IHF, and Xis (LR clonase). Then the desired sequence can be transferred to an expression vector by carrying out a one hour incubation at room temperature with Int, IHF, and Xis, a ten minute incubation at 37°C with proteinase K, transforming bacteria and allowing expression for one hour, and then plating on selective media. Generally, 90% cloning efficiency is achieved by this method. Examples of expression vectors are pDEST 14 bacterial expression vector with att7 promoter, pDEST 15 bacterial expression vector with a T7 promoter and a N-terminal GST tag, pDEST 17 bacterial vector with a T7 promoter and a N-terminal polyhistidine affinity tag, and pDEST 12.2 mammalian expression vector with a CMV promoter and neo resistance gene. These expression vectors or others like them are transformed or transfected into cells for expression of the target polypeptide or polypeptide variants. These expression vectors are often transfected, for example, into murine-transformed a adipocyte cell line 3T3-L1, (ATCC), human embryonic kidney cell line 293, and rat cardiomyocyte cell line H9C2.

[0265] Modifications may be made to the foregoing without departing from the basic aspects of the invention. Although the invention has been described in substantial detail with reference to one or more specific embodiments, those of skill in the art will recognize that changes may be made to the embodiments specifically disclosed in this application, yet these modifications and improvements are within the scope and spirit of the invention, as set forth in the claims which follow. All publications or patent documents cited in this specification are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference.

[0266] Citation of the above publications or documents is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents. U.S. patents and other publications referenced herein are hereby incorporated by reference.

#### Nucleotide and Amino Acid Sequence Embodiments

[0267] Table A includes information pertaining to the incident polymorphic variant associated with osteoarthritis identified herein. Public information pertaining to the polymorphism and the genomic sequence that includes the polymorphism are indicated. The genomic sequences identified in Table A may be accessed at the http address [www.ncbi.nih.gov/entrez/query.fcgi](http://www.ncbi.nih.gov/entrez/query.fcgi), for example, by using the publicly available SNP reference number (*e.g.*, rs756519). The chromosome position refers to the

position of the SNP within NCBI's Genome Build 34, which may be accessed at the following http address: [www.ncbi.nlm.nih.gov/mapview/map\\_search.cgi?chr=hum\\_chr.inf&query=](http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?chr=hum_chr.inf&query=). The "Contig Position" provided in Table A corresponds to a nucleotide position set forth in the contig sequence (see "Contig Accession No."), and designates the polymorphic site corresponding to the SNP reference number. The sequence containing the polymorphisms also may be referenced by the "Nucleotide Accession No." set forth in Table A. The "Sequence Identification" corresponds to cDNA sequence that encodes associated target polypeptides (e.g., ELP3). The position of the SNP within the cDNA sequence is provided in the "Sequence Position" column of Table A. If the SNP falls within an exon, the corresponding amino acid position (and amino acid change, if applicable) is provided as well. Also, the allelic variation at the polymorphic site and the allelic variant identified as associated with osteoarthritis is specified in Table A. All nucleotide and polypeptide sequences referenced and accessed by the parameters set forth in Table A are incorporated herein by reference.

**Table A**

RS_ID	Chromosome	Chrom Position	Contig Accession No. [1]	Contig Position	Nucleotide Accession No. [2]	Sequence Position	Amino Acid Position	Locus	Locus ID	A [3]	Allelic Variability	OA Assoc. Allele
rs756519	6	170707371	Hs6_7740_34:11	520890	NM_002793	intron		PSMB1	5689	F	[C/T]	T
rs1042327	6		does not map		NM_003194	exonic		TBP	6908	R	[T/C]	C
rs8770	6	170743040	Hs6_7740_34:11	556559	NM_002598	mma-utr		PDCD2	5134	R	[C/T]	C
rs1563055	8	27976377	Hs8_23822_34:16	6328752	NM_018091	intron		ELP3	55140	F	[C/T]	T
rs912428	13	44965904	Hs13_24680_34:1	28147904	NM_015116	intron		CHDC1	23143	R	[T/C]	T
rs1888475	21	38832863	Hs21_11669_34:9	25572863	NM_004449	intron		ERG	2078	F	[A/G]	A

[1] Contig Accession Number which can be found in the NCBI Database:

http address: [www.ncbi.nih.gov/entrez/query.fcgi](http://www.ncbi.nih.gov/entrez/query.fcgi)

[2] Sequence Identification or Nucleotide Accession Number which can be found in the NCBI Database:

http address: [www.ncbi.nih.gov/entrez/query.fcgi](http://www.ncbi.nih.gov/entrez/query.fcgi)

[3] "A" column is the sequence orientation ("F" is forward, "R" is reverse).

[0268] Following are genomic nucleotide sequences for a *chrom 6* region (SEQ ID NO: 1), an *ELP3* region (SEQ ID NO: 2), a *CHDC1* region (SEQ ID NO: 3) and an *ERG* region (SEQ ID NO: 4). The following nucleotide representations are used throughout: "A" or "a" is adenosine, adenine, or adenylic acid; "C" or "c" is cytidine, cytosine, or cytidylic acid; "G" or "g" is guanosine, guanine, or guanylic acid; "T" or "t" is thymidine, thymine, or thymidylic acid; and "I" or "i" is inosine, hypoxanthine, or inosinic acid. Exons are indicated in italicized lower case type, introns are depicted in normal text lower case

type, and polymorphic sites are depicted in bold upper case type. SNPs are designated by the following convention: "R" represents A or G, "M" represents A or C; "W" represents A or T; "Y" represents C or T; "S" represents C or G; "K" represents G or T; "V" represents A, C or G; "H" represents A, C, or T; "D" represents A, G, or T; "B" represents C, G, or T; and "N" represents A, G, C, or T.

chrom 6 genomic sequence (SEQ ID NO: 1)

>6:170689051-170779900

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121    tagagataaaa atctactgca gaaggcaaga cacttaactt ctgcccagaa actgatgtca
181    cgtgggttaac gtgtgggtggc cacatcaact gaagccgaca gtgacaccRa gtcagcattcc
241    tgggtgcagc tgccaccctg tcactctcat cacattccac cattcccagg ccacagaggc
301    aagaggatac tctcaaaact tagagatagc tttagtttta ggaaaatagg acggaaaaat
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1021   atcaaggtgt taaccatcag gagcagagaa aagagagtgt taagtatagg gccttagctg
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1741   cacctgtagt tccagctact aaggaggcta aggtgagagg accactgagc ccaggggacgt
1801   agagactacg gtgagacatg attataccac tgcactccag cctgggcaac agagggagac
1861   ctctctctcaa aaagaaaaga aaagaaaaag aaacaggtct aatttgttca tctaagcaat
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1981   agcctctaaa ttttaactgcc aatttatagg aaaagacagg acagaacctc caggaatgca
2041   atcattttata tctagaatgt ggaagattct gcatgacaaa cgacttggat tcttcaacat
2101   gtaaaatttca aggaaagaga gagagagaga gataaaaagg cttgtttcca gatttgaatg
2161   acatgttaat agacatttat gagacaaatca aggaaatttg aacatggact gcataattgaa
2221   gtgtgaggga ttatagttaa tttttaaagg tacaattgtg atactgtgat tatattttta
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90121	agtgcagagc	cagatgaggg	atggaaatca	gatctgccac	ttactccttg	gaaacctata
90181	aatgatcact	ttatcctcca	agagccttag	ttacacattt	gcacaatgag	gataWtatta
90241	ctgtagtata	ataagaactc	catttgccct	tgtctctcgt	ttctggcatg	gagctcctaa
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90601	tggtgcaacc	caatgctaaa	gggacagagg	cggccaggcg	cgggtggtca	cgctgtaat
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92401	gaaatggctY	atctttccaa	atgaataaaa	atacttatag	ctatgggttg	aattgtatca
92461	attctctccc	ccagttcatg	cactggagta	ctaaccoccg	gtccctcggg	atgtgatgat
92521	agagaggtag	ggcctttaca	gaggcaatca	aattaaagtg	aggtcattag	tgacctaac
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94261	acccttttac	tttgtaaatg	ctaggaggat	ctgtattttt	taaaatctta	gaacactaac
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94981	tattttatttt	ttaaaccaac	taataaaaagt	aatatatatt	taccgtgtac	aacattatgt
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95161	tttttttgtg	gtgcgaacac	ttgaaatcta	ctcttagtga	ttttcaagaa	tataatgcat
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97201	accctccaag	ccctgcccct	ggcagtgagg	acaaaaccaca	tgacccctgg	gattcagtat
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97321	ctggactgga	aatggccaaa	gctgaacaca	tctgagtacc	tgaagggtaa	gcttaagggtg
97381	tcaaagcagc	catgggtgagg	cagaaaagaac	cttgagcttg	gggccccaaag	tttatgctga
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97501	aagaggctct	gactccagag	agggtgtgca	agttcaatga	gaagggggtg	ctgatggtcc
97561	agatggacag	atgcaaagca	ctgctttctg	tttttgtctt	tgttttgata	caggattctc
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97741	atgcaccacc	atgcctggct	aatttttaaa	aaaaaaNttt	tggagacatg	agatctcact
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98461	agaggacttc	taggaaaagt	gttggctgat	gaagaaaagg	catagagtag	tagtcctgtc
98521	tgtgtggcac	ttggaactgt	gagagccatc			

[0269] Following are cDNA sequences for *PSMB1* (SEQ ID NO: 5), *TBP* (SEQ ID NO: 6), *PDCD2* (SEQ ID NO: 7 and 8), *ELP3* (SEQ ID NO: 9), *CHDC1* (SEQ ID NO: 10), and *ERG* (SEQ ID NO: 11 and 12).

*PSMB1* cDNA sequence (SEQ ID NO: 5)

NM\_002793 Homo sapiens proteasome (prosome, macropain) subunit, beta type, 1 (PSMB1), mRNA

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1 aaggcagcca tctcgccgtg agacagcaag tgtcgcgag ccgtgcgatg ttgtcctcta
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121 ctttgcagct gcgattttcg ccctacgttt tcaacggagg tactatactg gcaattgctg
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241 cgcgggtag ccccaaatgt tacaaattaa cagacaaaac agtcattgga tgcagcgggt
301 ttcattgaga ctgtcttacg ctgacaaaga ttattgaagc aagactaaag atgtataagc
361 attccaataa taaggccatg actacggggg caattgctgc aatgctgtct acaatcctgt
421 attcaaggcg cttctttcca tactatgttt acaacatcat cggtggaact gatgaagaag
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541 ctggaggctc agcaagtgcc atgctacagc ccctgcttga caaccagggt ggttttaaga
601 acatgcagaa tgtggagcat gttccgctgt ccttgagacag agccatgcgg ctggtgaaag
661 atgtcttcat ttctgcggt gagagagatg tgtacactgg ggacgcactc cggatctgca
721 tagtgaccaa agagggcatc agggaggaaa ctgtttcctt aagggaaggac tgatctgtgt
781 gctcttatca ccaatcagtt cagacctggt tgattttgta ctttggaaact gtaccttgga
841 tggttttgtt tattaaga gaaacctgaa gt

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*TBP* cDNA sequence (SEQ ID NO: 6)

NM\_003194 Homo sapiens TATA box binding protein (TBP), mRNA

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1 ggttcgtgt ggcggcgcc tgggcccgg gctgtttaac ttcgcttcg ctggcccata
61 gtgattttt cagtgacca gcagcatcac tgtttcttgg cgtgtgaaga taaccaagg
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301 cggaatccct atcttttagt caatgatgcc ttatggcact ggactgacct cacagcctat
361 tcagaacacc aatagtctgt ctattttgga agagcaacaa aggcagcagc agcaacaaca
421 acagcagcag cagcagcagc agcagcagca gcagcagcag cagcagcagc agcagcagca
481 gcagcagcag cagcagcagc agcagcagca gcaacaggca gtggcagctg cagccgttca
541 gcagtcaacg tcccagcagg caacacaggg aacctcaggc caggcaccac agctcttcca
601 ctacagact ctacaaactg cacccttgcc gggcaccact ccaactgtat cctccccat
661 gactcccatg acccccatca ctctgcccac gccagcttcg gagagtctg ggttgatcc
721 gcagctgcaa aatattgtat ccacagttaa tcttggttgt aaacttgacc taaagaccat
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1261 atgtaccctt gcctcccca ccccttctt tttttttttt taacaaatc agtttgtttt
1321 ggtaccttta aatggtggtg ttgtgagaag atggatgttg agttgcaggg tgtggacca
1381 ggtgatgccc ttctgtaagt gccaccgagg ggtatgcccgg aaggggcatt attgtgcac
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1501 tgccatttta tttatatgta gatttttaac actgctgttg acaagttggt ttgagggaga
1561 aaactttaag tgtaaagcc acctctataa ttgattggac tttttaattt taatgttttt
1621 ccccatgaac cacagttttt atatttctac cagaaaagta aaaatctttt taaaagtgt
1681 tgtttttcta atttataact cctaggggtt atttctgtgc cagacacatt ccacctctcc
1741 agtattgcag gacagaatat atgtgttaat gaaaatgaat ggctgtacat atttttttct

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1801 ttcttcagag tactctgtac aataaatgca gtttataaaa gtgttaaaaa aaaaaaaaaa  
1861 aaaaaaa

PDCD2 cDNA sequence 1 (SEQ ID NO: 7)

NM\_002598 Homo sapiens programmed cell death 2 (PDCD2), transcript variant 1, mRNA

1 tcttgcccttc cggcccgcg cccgatttcc gccttccgac ccagctgtgg gctgcgcccc  
61 acgccagccc gcgcccgcga tggctgccgc cggggccagg cctgtggagc tgggcttcgc  
121 cgagtcggcg ccggcgtggc gactgcgcag cgagcagttc cccagcaagg tgggcgggcg  
181 gccgcatgg ctgggcgcgg ccgggctgcc ggggccccag gccctggcct gcgagctgtg  
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421 gaatcctccc ccagaaacag gagaatcagt gtgtctccag cttaagtctg gtgctcatct  
481 ctgcagggtt tgtggctgtt taggccccaa aacgtgctcc agatgccaca aagcatatta  
541 ctgcagcaag gagcatcaga ccctagactg gagattggga cataagcagg cttgtgcaca  
601 accagatcat ctggaccata taattccaga ccacaacttc ctttttccag aatttgaaat  
661 tgtaatagaa acagaagatg agattatgcc tgaggttgtg gaaaaggaag attactcaga  
721 gattataggg agcatgggtg aagcacttga ggaagaactg gattccatgg caaaacatga  
781 atccagggaa gataaaattt ttcagaagtt taaaactcag atagcccttg aaccagaaca  
841 gattcttaga tatggcagag gtattgcccc catctggatt tctggtgaaa atattcctca  
901 agaaaaggat attccagatt gccctgtgg tgccaagaga atattggaat tccaggatcat  
961 gcctcagctc ctaaaactacc tgaaggctga cagactgggc aagagcattg actggggcat  
1021 cctggctgtc ttcacctgtg ctgagagctg cagcttgggt actggctata cagaagaatt  
1081 tgtgtggaag caggatgtaa cagatacacc gtaaaggcat cttaaagcct tgaaaaatgt  
1141 taataatctt ttataccttg caattccatt tctgggattt tatcctaagg aaatacttat  
1201 accaaaaata gaggtgcaga gatgttgaca gattgcttac acagtgtcta cttattagtg  
1261 aaacaaaagt gtccagtgcac agggaattaa ataaattttg gtacatccac a

PDCD2 cDNA sequence 2 (SEQ ID NO: 8)

NM\_144781 Homo sapiens programmed cell death 2 (PDCD2), transcript variant 2, mRNA

1 tcttgcccttc cggcccgcg cccgatttcc gccttccgac ccagctgtgg gctgcgcccc  
61 acgccagccc gcgcccgcga tggctgccgc cggggccagg cctgtggagc tgggcttcgc  
121 cgagtcggcg ccggcgtggc gactgcgcag cgagcagttc cccagcaagg tgggcgggcg  
181 gccgcatgg ctgggcgcgg ccgggctgcc ggggccccag gccctggcct gcgagctgtg  
241 cggccgcccg ctctccttcc tgctgcaggt gtatgcgccg ctgcctggcc gcccggaacgc  
301 cttccaccgc tgcattctcc tcttctgctg ccgcgagcag ccgtgctgtg ccggcctgcg  
361 agtttttagg aatcaactac ccaggaaaaa cgatttttac tcatatgagc caccttctga  
421 gaatcctccc ccagaaacag gagaatcagt gtgtctccag cttaagtctg gtgctcatct  
481 ctgcagggtt tgtggctgtt taggccccaa aacgtgctcc agatgccaca aagcatatta  
541 ctgcagcaag gagcatcaga ccctagactg gagattggga cataagcagg cttgtgcaca  
601 accagatcat ctggaccata taattccaga ccacaacttc ctttttccag aatttgaaat  
661 tgtaatagaa acagaagatg agattatgcc tgaggttgtg gaaaaggaag attactcaga  
721 gattataggg agcatgggtg agcagtttca ggacttcatt cattaagtgg ttaaacataa  
781 tacttggaag aaagggtcc atgtgcctag aagagaggta ctgagaggaa gactcacttt  
841 ggaggctgta gcatacaatt ttcagatatt gcctcaggta aaaatatact tcctggactt  
901 tgttttctga cacataagag gtgtgttctg ctccctgtaa agacaagggt gggtatccag  
961 atggtcccat gagtagggct gcacaagatg ctggaggctt ggtaagttcc tctgggtcgc  
1021 agatcggttt ctcggtcgg gatagtgtga gtgcctagca cagtgtcggg cacgcagaag  
1081 ggcccttaa aagtttctct ttcattctggc cagttttaga tacacaattt tgtcagttta  
1141 cttacagtgc atactcttgg gtagtacttg tgctgaccaa gtatcttaga ggcttatttt  
1201 tgtttctgag ccaacattta tccagcactt accttatata aagggtctgt tgtgcatgag  
1261 ctcatataaaa tcgtgacagc agaccaatga gtgagaaact gccccatttt gaagggtgag  
1321 aaattgaggt tctgggtata actttctttg gtcacataat attaaatttt acaatttgag  
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1441 agcttacttg tttgtagtgc ataccagtcac tacattccaa aacatgtttt gactcttact
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1561 gatgctcaca gtcttatgga ggagcccaaa taatatctgg ggaagttaaa gtccatataa
1621 tgactgataa gagtacaata caggtgccat gggaacacgt gacatcactg aagactgcct
1681 ggaagggggc gcgcgtgtgt tcatgcctat acgataaaca tgatacataa tgaaaatgct
1741 tatctttagg agaaaggaga gcctagagta gcaggatcaa ggatgaaagc tggacttcaa
1801 atatgccttg ttagtgtaaa tgtgactgtg gaactgtatg agtattttta gattatggag
1861 taaagtaagt tttaaaaagc agtccctaata catcaaaagt aaaaaactct tgatgtagtc
1921 atataaccac actaagaact cttccaggtg acttcaaaac ataggacagt acatctctag
1981 tagaatatgc cctgagaatg aaaagaatgt aacagtgtta gtattttgaa taaacatgtt
2041 attactaaaa aaaaaaaaaa aaaaaa

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# ELP3 cDNA sequence (SEQ ID NO: 9)

NM\_018091 Homo sapiens elongation protein 3 homolog (S. cerevisiae) (ELP3), mRNA

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1 gcagaaatga ggcagaagcg gaaaggagat ctcagccctg ctgagctgat gatgctgact
61 ataggagatg ttattaaaca actgattgaa gccacagagc aggggaaaga catcgatcta
121 aataaggtga aaaccaagac agctgccaaa tatggccttt ctgcccagcc ccgcctgggtg
181 gatatacttg ctgccgtccc tccctcagtat cgcaaggctc tgatgcccaa gttaaaggcg
241 aaacccatca gaactgctag tgggattgct gtcgtggctg tgatgtgcaa accccacaga
301 tgtccacaca tcagttttac aggaaatata tgtgtatact gccctgggtg acctgattct
361 gatttttgagt attccaccca gtcttacact ggctatgagc caacctccat gagagctatc
421 cgtgccagat atgacccttt cctacagaca agacaccgaa tagaacagtt aaaacaactt
481 ggtcatagtg tggataaagt ggagtttatt gtgatgggtg gaacgtttat ggcccttoca
541 gaagaatata gagattatgt tattcgaaat ttacatgatg ccttatcagg acatacttcc
601 aacaatattt acgaggcagt caagtattct gagagaagcc tcacaaagtg tattggaatt
661 actattgaaa ccagaccaga ttactgcatg aagcgacatt taagtgcatt gttgacctat
721 ggctgcacaa ggctggagat tgggggtgcag agtgtttatg aagatgtggc tagagacacc
781 aacagggggc acactgtgaa ggcagtgtgt gactcatttc acctggccaa agattccggg
841 tttaaagtgg tggcccatat gatgcctgac ctgccaaacg tgggactaga aagagacatt
901 gaacagttca cagagttttt tgagaaccct gcttttcgtc ccgatgggct gaaactctat
961 cctaccctgg tgattcgtgg gaccgggctt tatgagcttt ggaaatcagg aagatataag
1021 agttactctc ctagtgcact ggttgaattg gtggctcgga tcttagccct cgtgcctcca
1081 tggactcgag gtaccgagt acagagggat attccaatgc ctttagttag ctacaggata
1141 gagcatggta acctgagaga gctggcactt gcaagaatga aagacctcgg aatacagtgt
1201 cgagatgtga gaaccagaga agttggaatc caagaaattc atcacaaggt acggccatac
1261 caggttgaaat tggtaaggag agattatgtt gcaaatgggt gctgggaaac attcttgtca
1321 tacgaagacc cagatcaaga cattttgatt ggccctctac gattacgcaa gtgttcagaa
1381 gaaactttcc gtttcgaatt ggggtggagt gtctccatag tacgagagct gcatgtgtat
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1501 atgctgctga tggaggagc agaaagaata gctagagaag aacatgggtc tgggaaaatc
1561 gctgtgatat caggggtcgg caccaggaat tattatagaa agatcggcta cagattacaa
1621 ggcccgtaga tggatgaagt gctgaaataa tggccacacc agtccactct tctgcagtat
1681 cctccctggc agaacacgga gaatcaggat ttcttaataa ctcaacagag aggctgagca
1741 gagcaaatgg ggggcttcac cctcatcccg cagctgcaga gactggaaac tgccttcaag
1801 gccacggctg gtcatctgct gaccacaccc cagatccgcc ctctcctgcg tgcaccccaa
1861 aaaatcactt gcgtttttga ggcttaaatc atctatccag tttctacatt ttgcatgagg
1921 cctgcagggt gcctattttg actcagacgg tgaaaaaagc aaattaactc atttggacac
1981 cataactcat gcaataaaaac tgattgtcat tcgaggagca aacttaagag tagtttattt
2041 atataccctg gggacagaaa gtcagggtga aacaggaaaa ccaccagact ctaatctcag
2101 ccctttaacg acatacgcat tggagcgcaa gttaggaaaa tgagcttttg tttcatgga
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2221 aattgcagtt ctgggggtgt gagcctggca gcagccagcc agcagcctct ccaggcggg
2281 agtctacatc ccgagacggc gatgacaagc agcttcattc cacattcttt gttatctcta
2341 ctccccacc tcttggaac tacagagcag tgtgggcagc cccaagtgtg gtccccagag
2401 agcgtttggc tttcctgtct gtctatcctg agcgggtgga gtctcaggtt gtgtgccctt
2461 aaatcaagat ttgcttcac agaagccatt acttgcaatt tttttttttt tttctgagaa
2521 agtctcgctg tgtcaccag gctggagtgc agtggcgcaa tctcactgca tctccgctc
2581 cccgggttca agcagattct cgcctcagc ctctgagta gctgggatta caggcacccg
2641 ccgctgctaa tttttgtatt tttagtagag atgggggttt caccatattg gtcaggctgg
2701 tctcgaactc ctgacctcag gtgatcaacc caccttggcc tccctaaatg ccgggattac

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2761 aggcattgagc caccgctccc agcctttgat tttttaaggt ggatttttggg tgttataaat
2821 ggagaaaggt aagagttcaa gttcaacccg tgtgtgaaaag caaaacaatg gaaaacagga
2881 ttggcttctt caaaggctcc tctttagtaa ctgctctttt gaaatttcga ggtaattctac
2941 tttggagact ctgcttgag agggctcagtt cctaagttaa aagcatcgct taaccttggc
3001 tcctgtggca ttttacaagg gtttaagga attgattcct ctgaaagggc ctgaaaataa
3061 aaagtcttta acatataaaa aaaaaaaaaa aaaaa

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CHDC1 cDNA sequence (SEQ ID NO: 10)

NM\_015116 Homo sapiens calponin homology (CH) domain containing 1 (CHDC1), mRNA

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1  ccgcagtcct tagcttcccc gggacaggaa accttcaaga ccgagctgcc acggccgcct
61  ccccgccgcg ccccattct acgcgcctgc ccacaccctc ctcccctcct tccagcgcct
121  ttcggtggag cactgcggca ctacgcccga gctgcctgtt tcccctcgcg gggaacgctg
181  tgaccccccc gcaggagcgg cggggcgggg tggggggggc cgggagaaga tggcgacgcc
241  gggaagcgaa ccccaacctt tcgtcccggc cctttcggtg gctactctgc acccacttca
301  tcatccccac caccaccacc accaccatca gcaccacgga ggaaccggcg cccccggcgg
361  ggcgggtggt ggcggcggtg gcagcggggg cttcaacctg cccttgaacc ggggtctgga
421  gcgcgcgctt gaggagcgcg ccaactccgg ggggctgaac ctgagcgcca ggaaattgaa
481  ggaatttccc cgtaccgcag cccccgggca tgacctctcg gacacggtgc aggcagactt
541  atctaaaaac agactggttg aagttccaat ggaattgtgc cattttgtat cactggaaat
601  tcttaattctg tatcacaact gtatcagagt cattcctgag gccatcgtaa atctgcagat
661  gctgacttac ctgaacttga gtcgaaatca ctggtccgcc ctgctgcctt gcctgtgtgg
721  tctgcctctc aaagtcttaa tcgcaagtaa caacaaactt ggatcattac cagaagagat
781  aggtcagctc aaacagttaa tggagctgga tgctcagctg aacgagatca cagcgttgcc
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901  agttttacca caagaactag tagatcttcc cttggtaaag tttgactttt cctgcaacaa
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1021  gaataaccct ctgcagtctc ctccagcaca gatttgcaca aagggcaag ttcacataat
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1321  ctggtgccat cgccttagcc ccgttaaagg ggaatttcat cagggaattc aaccggagcc
1381  ttcccttttg ggtgacagca ccaactcagg agaagaaaga gaccagttaa ctgatagagc
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1561  tcaggacatg gatatagcaa tgatcgagca gctgagagaa gctgcaaga tgctgcaaga
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1681  atcagcagaa gccttagaat tacaagattc tgcactgaat ggtcaaatac agctggagac
1741  atctccggtg tgtgaggtgc aaagtgatct aacattacag agtaacggga gccagtatcc
1801  tccaaatgag attagagaga actcccctgc agtctctcct accacaaaca gcacagctcc
1861  atttggcctg aagcctcgat cagtgtttct aagacctcag agaaatttgg aatctataga
1921  cccgcagttt acaatccgga ggaaaatgga gcagatgaga gaagagaaag agctggtgga
1981  acaacttcgt gagagcattg agatgagatt gaaggtcagt ctacacgaag acctgggggc
2041  agccctcatg gatggtgtcg tcctctgcca tctggtcaac cacatccgcc cagggtcggt
2101  tgcaagcatc catgtcccat caccagcggg tcccaaactt agcatggcca aatgcagaag
2161  aaatgtggaa aactttttgg aagcgtgccg aaaattagga gtaccagagg ctgacctctg
2221  ctctccgtgt gacatcctgc agttggattt tcgtcacatt cgaaagactg ttgacactct
2281  gctggcactc ggggagaaag cccaccacc aacttctgcc ctccgctcca gggaccttat
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2521  ttcagttatc tctcgagttt tgaagctgaa cagtagcaaa tcagattttc cagaagcaca
2581  aactttgtag aatacagttt agtataattc ctctcactta ctgaaatata acgacgacga
2641  ctgcaaagtg tatgcacacc gcatgcttcc tcatccacat agtgccagca gcagtgccac
2701  gcagttcctc ctctccctcc cggtagagct ctgccctggg cagagggggg gagaattcca
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2821  gaactagaat tctaattcgg gactgggcaa ttgagctgta taggggccac cttgcaggga
2881  ggacagaaaa ctaacatttt ggcccaactt gatctatata aaactttaat aataccacta
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3001 ctcccaaatt tcttaaaaag ggagccgcga agggcgctgg gcagtgtggc cgccaacttc
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3961 gactttgtgt tgattgtgta aatgcagggt atgtggaagt tatctaatta acctcagttg
4021 tatatgaata acccacagat gtactgaatt acttttggtg ctatcttgta ctcttcaatc
4081 tgtaacacaa taaaatccct ttgtacgatg tctaattgagc accctgagcc ataaattgct
4141 taataaacac attttgggtg att

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ERG cDNA sequence 1 (SEQ ID NO: 11)

NM\_182918 Homo sapiens v-ets erythroblastosis virus E26 oncogene like (avian), (ERG), transcript variant 1, mRNA

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1 aatctcatcc gctctaaaca acctcatcaa aactactttc tggtcagaga gaagcaataa
61 ttattattaa catttattaa cgatcaataa acttgattgc attatggcca gcactattaa
121 ggaagcctta tcagttgtga gtgaggacca gtcgttggtt gagtgtgcct acggaacgcc
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361 tgtggccaaa ggcggaaga tgggtggcag cccagacacc gttgggatga actacggcag
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661 catccttctc tcacatctcc actacctcag agagactcct ctccacatt tgacttcaga
721 tgatgttgat aaagccttac aaaactctcc acggttaatg catgctagaa acacaggggg
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841 taggccagat ttaccatatg agccccccag gagatcagcc tggaccggtc acggccaccc
901 cagcccccag tcgaaagctg ctcaaccatc tcttccaca gtgccccaaa ctgaagacca
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1381 gaagatgaac tttgtggcgc cccacctcc agccctccc gtgacatctt ccagtttttt
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1681 agatccaaag actcttggga gggagtact gaagtcttac tacagaaatg aggaggtatg
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1861 acttttagagt agagtttgga atcccactaa tgcaaactgg gatgaaacta aagcaataga
1921 aacaacacag ttttgacctt acataccgtt tataatgcc a ttttaaggaa aactacctgt
1981 atttaaaaat agaaacatat caaaaaaaaa aaaaaa

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ERG cDNA sequence 2 (SEQ ID NO: 12)

NM\_004449 Homo sapiens v-ets erythroblastosis virus E26 oncogene like (avian), (ERG), transcript variant 2, mRNA

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1 atgattcaga ctgtcccga cccagcagct catatcaagg aagccttatt agttgtgagt
61 gaggaccagt cgttggttga gtgtgcctac ggaacgccac acctggctaa gacagagatg
121 accgcgtcct cctccagcga ctatggacag acttccaaga tgagcccacg cgtccctcag
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241 caggtgaatg gctcaaggaa ctctcctgat gaatgcagtg tggccaaagg cgggaagatg
301 gtgggcagcc cagacaccgt tgggatgaac tacggcagct acatggagga gaagcacatg
361 ccacccccaa acatgaccac gaacgagcgc agagttatcg tgccagcaga tcctacgcta
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481 gacgtcaaca tcttggttatt ccagaacatc gatgggaagg aactgtgcaa gatgaccaag
541 gacgacttcc agaggctcac cccagctac aacgccgaca tccttctctc acatctccac
601 tacctcagag agactcctct tccacatttg acttcagatg atgttgataa agccttacia
661 aactctccac ggtaaatgca tgctagaaac acagatttac catatgagcc cccaggaga
721 tcagcctgga ccggtcacgg ccaccccacg cccagtcga aagctgctca accatctcct
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1261 ctccccgtga catcttccag tttttttgct gcccacaaac catactggaa ttcaccaact
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1381 tactactaa

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[0270] Following are amino acid sequences for *PSMB1* (SEQ ID NO: 13), *TBP* (SEQ ID NO: 14), *PDCD2* (SEQ ID NO: 15 and 16), *ELP3* (SEQ ID NO: 17), *CHDC1* (SEQ ID NO: 18), and *ERG* (SEQ ID NO: 19 and 20).

*PSMB1* amino acid sequence (SEQ ID NO: 13)

NP\_002784 Homo sapiens proteasome (prosome, macropain) subunit, beta type, 1 (PSMB1), protein

MLSSTAMYSAPGRDLGMEPHRAAGPLQLRFSPYVFNGGTILAIAGEDFAIVASDTRLSE  
GFSIHTRDSPKCYKLTDKTVIGCSGFHGDCLTLTKIIEARLKMYSNNKAMTTGAIAA  
MLSTILYSRRFFPYVYNIIGGLDEEGKGAVYSFDPVGSYQRDSFKAGGSASAMLQPLL  
DNQVGFKNMQNVEHVPLSLDRAMRLVKDVFISAAERDVYTGDALRICIVTKEGIREET  
VSLRKD

*TBP* amino acid sequence (SEQ ID NO: 14)

NP\_003185 Homo sapiens TATA box binding protein (TBP), protein

MDQNNSLPPYAQGLASPQGAMTPGIPIFSPMMPYGTGLTPQPIQNTNSLSILEEQQRQQQ  
QQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQVAAAQVQSTSQQATQGT  
SGQAPQLFHSQTLTAPLPGTTPLYPSPMTPMTPITPATPASESSGIVPQLQNIVSTVNLG

CKLDLKTIALRARNAEYNPKRFAAVIMRIREPRTTALIFSSGKMVCTGAKSEEQSRLAAR  
KYARVVQKLGFPKFLDFKIQNMVGS CDVKFPIRLEGLVLTHQQFSSYEPELFPGLIYR  
MIKPRIVLLIFVSGKVVL TGAKVRAEIYEA FENIYPILKGFRKTT

PDCD2 amino acid sequence 1 (SEQ ID NO: 15)

NP\_002589 Homo sapiens programmed cell death 2 (PDCD2), isoform 1, protein

MAAAGARPVELGFAESAPAWRLRSEQFPSKVGGPRAWLGAAGLP GPQALACELCGRP  
LSFLLQVYAPLPGRPDAFHRCIFLCCREQPCCAGLRVFRNQLPRKNDFY SYEPPSENPPP  
ETGESVCLQLKSGAHL CRVCGCLGPKTCSRCHKAYYCSKEHQTLDWRLGHKQACAQP  
DHL DHIIPDHNFLFPEFEIVIETED EIMPEVVEKEDYSEIIGSMGEALEEEELDSMAKHESRE  
DKIFQKFKTQIALEPEQILRYGRGI APIWISGENIPQEKDIPDCPCGAKRILEFQVMPQLLN  
YLKADRLGKSIDWGILAVFTCAESCSLGTGYTEEFVWKQDVTDP

PDCD2 amino acid sequence 2 (SEQ ID NO: 16)

NP\_659005 Homo sapiens programmed cell death 2 (PDCD2), isoform 2, protein

MAAAGARPVELGFAESAPAWRLRSEQFPSKVGGPRAWLGAAGLP GPQALACELCGRP  
LSFLLQVYAPLPGRPDAFHRCIFLCCREQPCCAGLRVFRNQLPRKNDFY SYEPPSENPPP  
ETGESVCLQLKSGAHL CRVCGCLGPKTCSRCHKAYYCSKEHQTLDWRLGHKQACAQP  
DHL DHIIPDHNFLFPEFEIVIETED EIMPEVVEKEDYSEIIGSMGKQFQDFIH

ELP3 amino acid sequence (SEQ ID NO: 17)

NP\_060561 Homo sapiens elongation protein 3 homolog (S. cerevisiae) (ELP3), protein

MRQKRKGDLSPAELMMLTIGDVIKQLIEAHEQGKDIDL NKVKTKTAAKYGLSAQPRLV  
DIIAAVPPQYRKVLMPKLKAKPIRTASGIAVVAVMCKPHRCPHISFTGNICVYCPGGPDS  
DFEYSTQSYTGYEPTSMRAIRARYDPFLQTRHRIEQLKQLGHSVDKVEFIVMGGTFMAL  
PEEYRDYFIRNLHDALSGHTSNNIYEAVKYSERSLTKCIGITIETRPDYCMKRHLSDMLT  
YGCTRLEIGVQSVYEDVARDTNRGHTVKAVCESFH LAKDSGFKVVAHMMPDLPNVGL  
ERDIEQFTEFFENPAFRPDGLKLYPTLVIRGTGLYELWKSGRYKSYSPSDLVELVARILA  
LVPPWTRVYRVQRDIPMLVSSGVEHGNLRELALARMKDLGIQCRDVRTREVG IQEIIH  
KVRPYQVELVRRDYVANGGWETFLSYEDPDQDILIGLLRLRKCEETFRFELGGGV SIV  
RELHVGSVVPVSSRDPTKFQHQGF GMLLMEEAERIAREEHSGSGKIAVISGVGTRNYYR  
KIGYRLQGPYMKMLK

CHDC1 amino acid sequence (SEQ ID NO: 18)

NP\_055931 Homo sapiens calponin homology (CH) domain containing 1 (CHDC1), protein

MATPGSEPQPFVPAALSVATLHPLHHPHHHHHHHHQHGGGTGAPGGAGGGGGGGSGGFNL  
PLNRGLERALEEAANSGLNLSARKLKEFPRTAAPGHDLSDTVQADLSKNRLVEVPME  
LCHFVSLEILNLYHNCIRVIPEAIVNLQMLTYLNLSRNQLSALPACLCGLPLKVLIASNNK  
LGSLPEEIGQLKQLMELDVSCNEITALPQQIGQLKSLRELNVRNRYLKVLPQELVDLPLV  
KFDFSCNKVLVIPICFREMQLQVLLLENNPLQSPPAQICTKGKVHIFKYLSIQACQIKTA  
DSLYLHTMERPHLHQHVEDGKKDSDSGVGSNDGDKRLSATEPSDEDTVSLNVPMSNIM  
EEEQIIKEDSCHRLSPVKGEFHQEFQPEPSLLGDSTNSGEERDQFTDRADGLHSEFMNYK  
ARAEDCEELLRIEEDVHWQTEGISSSKDQDMDIAMIEQLREAVDLLQDPNGLSTDITER  
SVLNLYPMGSAEALQLQDSALNGQIQLETSPVCEVQSDTLQSNQSQYSPNEIRENSPAV  
SPTTNSTAPFGLKPRSVFLRPQRNLESIDPQFTIRRKMEQMREEKELVEQLRESIEMRLKV  
SLHEDLGAALMDGVVLCHLVNHIRPRSVASIHVPSPAVPKLSMAKCRNVENFLEACR  
KLGVPADLCSPCDILQLDFRHIRKTVDTLLALGEKAPPPTSALRSRDLIGFCLVHILFIVL  
VYITYHWNALSA

ERG amino acid sequence 1 (SEQ ID NO: 19)

NP\_891548 Homo sapiens v-ets erythroblastosis virus E26 oncogene like (avian), (ERG), isoform 1, protein

MASTIKEALSVVSEDQSLFECAYGTPHLAKTEMTASSSSDYGQTSKMSPRVPQQDWLS  
QPPARVTIKMECNPSQVNGSRNSPDECSVAKGGKMVGSPDTVGMNYGSYMEEKHMPP  
PNMTTNERRVIVPADPTLWSTDHVRQWLEWAVKEYGLPDVNILLFQNIIDGKELCKMT  
KDDFQRLTPSYNADILLSHLHYLRETPLPHLTSSDDVDKALQNSPRLMHARNTGGAAFIF  
PNTSVYPEATQRITTRPDLPEPPRRSAWTGHGHPTPQSKAAQSPSTVPKTEDQRPQLD  
PYQILGPTSSRLANPGSGQIQLWQFLLELLSDSSNSSCITWEGTNGEFKMTDPDEVARRW  
GERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKFDHFGIAQALQPHPPESSLY  
KYPSDLPYMGSYHAHPQKMNFVAPHPALPVTSSSFFAAPNPYWNSTGGIYPNTRLPT  
SHMPSHLGTYT

ERG amino acid sequence 2 (SEQ ID NO: 20)

NP\_004440 Homo sapiens v-ets erythroblastosis virus E26 oncogene like (avian), (ERG), isoform 2, protein

MIQTVDPDAAHIKEALSVVSEDQSLFECAYGTPHLAKTEMTASSSSDYGQTSKMSPRVP  
QQDWLSQPPARVTIKMECNPSQVNGSRNSPDECSVAKGGKMVGSPDTVGMNYGSYME  
EKHMPPPNMTTNERRVIVPADPTLWSTDHVRQWLEWAVKEYGLPDVNILLFQNIIDGKE  
LCKMTKDDFQRLTPSYNADILLSHLHYLRETPLPHLTSDDDVDKALQNSPRLMHARNTD  
LPYEPPRRSAWTGHGHPTPQSKAAQSPSTVPKTEDQRPQLDPYQILGPTSSRLANPGSG  
QIQLWQFLLELLSDSSNSSCITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSR  
ALRYYYDKNIMTKVHGKRYAYKFDFHGIAQALQPHPPESSLYKYPDLPYMGSYHAHP  
QKMNFVAPHPPALPVTSSSFFAAPNPYWNSPTGGIYPNTRLPTSHMPSHLGTYY

**[0271]** Modifications may be made to the foregoing without departing from the basic aspects of the invention. Although the invention has been described in substantial detail with reference to one or more specific embodiments, those of skill in the art will recognize that changes may be made to the embodiments specifically disclosed in this application, yet these modifications and improvements are within the scope and spirit of the invention, as set forth in the aspects which follow. All publications or patent documents cited in this specification are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference.

**[0272]** Citation of the above publications or documents is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents. U.S. patents and other publications referenced herein are hereby incorporated by reference.

What is claimed is:

1. A method for identifying a subject at risk of osteoarthritis, which comprises detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in a nucleic acid sample from a subject, wherein the one or more polymorphic variations are detected in a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence in SEQ ID NO: 1-12;

(b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(d) a fragment of a nucleotide sequence of (a), (b), or (c);

whereby the presence of the polymorphic variation is indicative of the subject being at risk of osteoarthritis.

2. The method of claim 1, which further comprises obtaining the nucleic acid sample from the subject.

3. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 170719500 to 170766500 in human genomic DNA.

- 4. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 1 selected from the group consisting of 229, 6310, 11840, 11870, 12064, 13392, 16354, 16559, 16935, 17616, 17737, 18321, 18453, 18811, 20020, 21662, 23197, 23446, 24339, 25504, 27174, 28008, 29294, 29759, 30832, 44512, 44850, 45884, 46345, 48589, 53371, 53911, 53990, 55152, 55667, 58952, 59315, 60029, 61477, 62988, 63090, 64021, 65685, 70220, 70323, 70959, 73436, 82945, 82958, 82961, 82964, 82965, 83006, 83025, 83034, 83074, 83132, 83155, 83172, 83174, 83206, 83216, 83234, 83252, 83260, 83263, 83296, 83319, 83322, 83324, 83357, 83375, 83381, 83389, 83443, 83499, 83545, 83566, 83591, 83619, 83698, 83780, 83784, 83826, 83832, 83852, 86297, 86315, 86420, 86460, 86714, 86718, 86736, 86753, 86766, 88162, 88218, 88246, 88255, 88309, 88310, 88471, 88619, 88904, 89044, 90531, 90534, 90613 and 46252.

5. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 1 selected from the group consisting of 229, 6310, 16559, 18453, 25504, 27174, 30832, 44850, 45884, 48589, 61477, 82961 and 46252.

6. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 27963000 to 27983000 in human genomic DNA.

7. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 2 selected from the group consisting of 211, 473, 1536, 5639, 17186, 17335, 25029, 25111, 28811, 28863, 30809, 40985, 45147, 45282, 46168, 46328, 49077, 51925, 52141, 52168, 60852, 62468, 65572, 79089, 79541, 79790, 90843, 90978, 91052, 91131, 91132, 94439 and 94621.

8. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 2 selected from the group consisting of 40985, 46168, 51925 and 52168.

9. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 44962000 to 45013000 in human genomic DNA.

10. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 3 selected from the group consisting of 243, 10208, 15049, 15111, 15272, 15287, 15326, 15327, 17038, 19391, 21702, 22431, 22881, 27744, 32564, 32698, 33104, 33181, 33256, 33543, 35567, 40085, 40482, 45641, 46059, 48504, 48919, 49693, 49874, 50020, 50616, 50719, 55511, 65533, 70529, 75591, 77266, 80368, 82475, 92462, 92480, 95819 and 96275.

11. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 3 selected from the group consisting of 15111, 45641, 46059, 49693, 49874, 50020, 50719, 70529, 82475, 92462, 92480 and 96275.

12. The method of claim 1, wherein the one or more polymorphic variations are detected within a region spanning chromosome positions 38830000 to 38844000 in human genomic DNA.

13. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 4 selected from the group consisting of 231, 882, 960, 1194, 1530, 1673, 2096, 2285, 5873, 7256, 7988, 8222, 8381, 8814, 8915, 9642, 9902, 10619, 10927, 11032, 14377, 15608, 15928, 16296, 17598, 19272, 20084, 20577, 28051, 29466, 29530, 29987, 30012, 30322, 32216, 32516, 32544, 32746, 33137, 33538, 33798, 33802, 33964, 34132, 34210, 34317, 34499, 34753, 34845, 35335, 36423, 36450, 36481, 38447, 38784, 39387, 39458, 39822, 40305, 40869, 40926, 41010, 41134,

41984, 42172, 42753, 43011, 43176, 43320, 43381, 44142, 44383, 44726, 45087, 45141, 45359, 45421, 45456, 45467, 45486, 45709, 45716, 47626, 49413, 49796, 49962, 50075, 50093, 50571, 50615, 50780, 50851, 51459, 53193, 53702, 53736, 53795, 54109, 54126, 54230, 54894, 55455, 55499, 56522, 56662, 56954, 57267, 58282, 58916, 59544, 59666, 59913, 66846, 67245, 67652, 67955, 67966, 68420, 70226, 70810, 72246, 73330, 73457, 74389, 74638, 74640, 75358, 75952, 76098, 77836, 78449, 78507, 80031, 81695, 82775, 82795, 84611, 84657, 84693, 85020, 85048, 85100, 85325, 85452, 85868, 85936, 85990, 86139, 86497, 87236, 87248, 87533, 87912, 88108, 88494, 89598, 90235, 91287, 91359, 92384, 92410, 92900, 94495, 94512, 97777 and 98333.

14. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in SEQ ID NO: 4 selected from the group consisting of 1673, 20577, 33137, 39822, 45716, 49962, 51459, 54894, 55455, 55499, 58282, 68420 and 80031.

15. The method of claim 1, wherein the one or more polymorphic variations are detected at one or more positions in linkage disequilibrium with one or more positions in claim 4, 7, 10 or 13.

16. The method of claim 1, wherein detecting the presence or absence of the one or more polymorphic variations comprises:

hybridizing an oligonucleotide to the nucleic acid sample, wherein the oligonucleotide is complementary to a nucleotide sequence in the nucleic acid and hybridizes to a region adjacent to the polymorphic variation;

extending the oligonucleotide in the presence of one or more nucleotides, yielding extension products; and

detecting the presence or absence of a polymorphic variation in the extension products.

17. The method of claim 1, wherein the subject is a human.

18. The method of claim 17, wherein the subject is a human female.

19. The method of claim 17, wherein the subject is a human male.

20. A method for identifying a polymorphic variation associated with osteoarthritis proximal to an incident polymorphic variation associated with osteoarthritis, which comprises:

identifying a polymorphic variation proximal to the incident polymorphic variation associated with osteoarthritis, wherein the polymorphic variation is detected in a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence in SEQ ID NO: 1-12;

(b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation;

determining the presence or absence of an association of the proximal polymorphic variant with osteoarthritis.

21. The method of claim 20, wherein the incident polymorphic variation is at one or more positions in claim 4, 7, 10 or 13.

22. The method of claim 20, wherein the proximal polymorphic variation is within a region between about 5 kb 5' of the incident polymorphic variation and about 5 kb 3' of the incident polymorphic variation.

23. The method of claim 20, which further comprises determining whether the proximal polymorphic variation is in linkage disequilibrium with the incident polymorphic variation.

24. The method of claim 20, which further comprises identifying a second polymorphic variation proximal to the identified proximal polymorphic variation associated with osteoarthritis and determining if the second proximal polymorphic variation is associated with osteoarthritis.

25. The method of claim 24, wherein the second proximal polymorphic variant is within a region between about 5 kb 5' of the incident polymorphic variation and about 5 kb 3' of the proximal polymorphic variation associated with osteoarthritis.

26. An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence in SEQ ID NO: 1-12;

(b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation; and

(e) a nucleotide sequence complementary to the nucleotide sequences of (a), (b), (c), or (d);

wherein the nucleotide sequence comprises a polymorphic variation associated with osteoarthritis selected from the group consisting of in SEQ ID NO: 1 a thymine at position 229, a guanine at position 6310, a thymine at position 16559, an adenine at position 18453, an adenine at position 25504, an adenine at position 27174, an adenine at position 30832, a guanine at position 44850, an adenine at position 45884, an adenine at position 48589, a cytosine at position 61477, a cytosine at position 82961 and a thymine at position 46252; in SEQ ID NO: 2 a cytosine at position 40985, a guanine at position 46168, a thymine at position 51925 and a cytosine at position 52168; in SEQ ID NO: 3 a guanine at position 15111, a thymine at position 45641, an adenine at position 46059, a cytosine at position 49693, an adenine at position 49874, an adenine at position 50020, a guanine at position 50719, an adenine at position 70529, an adenine at position 82475, a thymine at position 92462, a thymine at position 92480 and a cytosine at position 96275; and in SEQ ID NO: 4 a guanine at position 1673, a thymine at position 20577, a guanine at position 33137, a guanine at position 39822, an adenine at position 45716, a guanine at position 49962, an adenine at position 51459, a cytosine at position 54894, an adenine at position 55455, an adenine at position 55499, a guanine at position 58282, an adenine at position 68420 and a thymine at position 80031.

27. An oligonucleotide comprising a nucleotide sequence complementary to a portion of the nucleotide sequence of (a), (b), (c), or (d) in claim 26, wherein the 3' end of the oligonucleotide is adjacent to a polymorphic variation associated with osteoarthritis.

28. A microarray comprising an isolated nucleic acid of claim 26 linked to a solid support.

29. An isolated polypeptide encoded by the isolated nucleic acid sequence of claim 26.

30. A method for identifying a candidate therapeutic for treating osteoarthritis, which comprises:

(a) introducing a test molecule to a system which comprises a nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(i) a nucleotide sequence in SEQ ID NO: 1-12;

(ii) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(iii) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(iv) a fragment of a nucleotide sequence of (a), (b), or (c); or

introducing a test molecule to a system which comprises a protein encoded by a nucleotide sequence of (i), (ii), (iii), or (iv); and

(b) determining the presence or absence of an interaction between the test molecule and the nucleic acid or protein,

whereby the presence of an interaction between the test molecule and the nucleic acid or protein identifies the test molecule as a candidate therapeutic for treating osteoarthritis.

31. The method of claim 30, wherein the system is an animal.

32. The method of claim 30, wherein the system is a cell.

33. The method of claim 30, wherein the nucleotide sequence comprises one or more polymorphic variations associated with osteoarthritis.

34. The method of claim 33, wherein the one or more polymorphic variations associated with osteoarthritis are at one or more positions in claim 4, 7, 10 or 13.

35. A method for treating osteoarthritis in a subject, which comprises contacting one or more cells of a subject in need thereof with a nucleic acid, wherein the nucleic acid comprises a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence in SEQ ID NO: 1-12;

(b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(d) a fragment of a nucleotide sequence of (a), (b), or (c); and

(e) a nucleotide sequence complementary to the nucleotide sequences of (a), (b), (c), or (d);

whereby contacting the one or more cells of the subject with the nucleic acid treats the osteoarthritis in the subject.

36. The method of claim 35, wherein the nucleic acid is RNA or PNA.

37. The method of claim 36, wherein the nucleic acid is duplex RNA.

38. A method for treating osteoarthritis in a subject, which comprises contacting one or more cells of a subject in need thereof with a protein, wherein the protein is encoded by a nucleotide sequence which comprises a polynucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence in SEQ ID NO: 1-12;

(b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(d) a fragment of a nucleotide sequence of (a), (b), or (c);

whereby contacting the one or more cells of the subject with the protein treats the osteoarthritis in the subject.

39. A method for treating osteoarthritis in a subject, which comprises:

detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in a nucleic acid sample from a subject, wherein the one or more polymorphic variation are detected in a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence in SEQ ID NO: 1-12;

(b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation; and

administering an osteoarthritis treatment to a subject in need thereof based upon the presence or absence of the one or more polymorphic variations in the nucleic acid sample.

40. The method of claim 39, wherein the one or more polymorphic variations are detected at one or more positions in claim 4, 7, 10 or 13.

41. The method of claim 39, wherein the treatment is selected from the group consisting of administering a corticosteroid, a nonsteroidal anti-inflammatory drug (NSAID), a cyclooxygenase-2 (COX-2) inhibitor, an antibody, a glucocorticoid, hyaluronic acid, chondrotin sulfate, glucosamine or acetaminophen; prescribing a heat/cold regimen or a joint protection regimen; performing joint surgery; prescribing a weight control regimen; and combinations of the foregoing.

42. A method for detecting or preventing osteoarthritis in a subject, which comprises:

detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in a nucleic acid sample from a subject, wherein the polymorphic variation is detected in a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence in SEQ ID NO: 1-12;

(b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12;

(d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation; and

administering an osteoarthritis prevention or detection procedure to a subject in need thereof based upon the presence or absence of the one or more polymorphic variations in the nucleic acid sample.

43. The method of claim 42, wherein the one or more polymorphic variations are detected at one or more positions in claim 4, 7, 10 or 13.

44. The method of claim 42, wherein the osteoarthritis prevention is selected from the group consisting of administering a corticosteroid, a nonsteroidal anti-inflammatory drug (NSAID), a cyclooxygenase-2 (COX-2) inhibitor, an antibody, a glucocorticoid, hyaluronic acid, chondrotin sulfate, glucosamine or acetaminophen; prescribing a heat/cold regimen or a joint protection regimen; performing joint surgery; prescribing a weight control regimen; and combinations of the foregoing.

45. A method of targeting information for preventing or treating osteoarthritis to a subject in need thereof, which comprises:

detecting the presence or absence of one or more polymorphic variations associated with osteoarthritis in a nucleic acid sample from a subject, wherein the polymorphic variation is detected in a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence in SEQ ID NO: 1-12;
- (b) a nucleotide sequence which encodes a polypeptide encoded by a nucleotide sequence in SEQ ID NO: 1-12;
- (c) a nucleotide sequence which encodes a polypeptide that is 90% or more identical to the amino acid sequence encoded by a nucleotide sequence in SEQ ID NO: 1-12;
- (d) a fragment of a nucleotide sequence of (a), (b), or (c) comprising a polymorphic variation; and

directing information for preventing or treating osteoarthritis to a subject in need thereof based upon the presence or absence of the one or more polymorphic variations in the nucleic acid sample.

46. The method of claim 45, wherein the one or more polymorphic variations are detected at one or more positions in claim 4, 7, 10 or 13.

47. A composition comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and an antibody that specifically binds to a protein, polypeptide or peptide encoded by a nucleotide sequence identical to or 90% or more identical to a nucleotide sequence in SEQ ID NO: 1-12.

48. A composition comprising a cell from a subject having osteoarthritis or at risk of osteoarthritis and a RNA, DNA, PNA or ribozyme molecule comprising a nucleotide sequence identical to or 90% or more identical to a portion of a nucleotide sequence in SEQ ID NO: 1-12.

49. The composition of claim 48, wherein the RNA molecule is a short inhibitory RNA molecule.

Abstract of the Disclosure

Provided herein are methods for identifying a risk of osteoarthritis in a subject, reagents and kits for carrying out the methods, methods for identifying candidate therapeutics for treating osteoarthritis, and therapeutic and preventative methods applicable to osteoarthritis. These embodiments are based upon an analysis of polymorphic variations in nucleotide sequences within the human genome.

FIGURE 1A

CHROM 6 – DISCOVERY P-VALUES (female only)

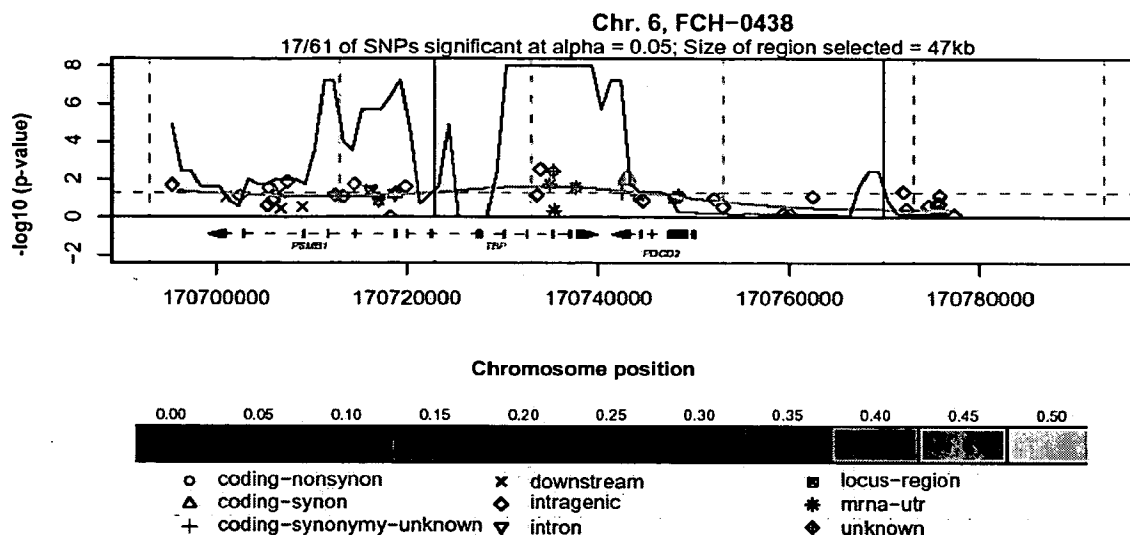


FIGURE 1B

ELP3 – DISCOVERY P-VALUES (female only)

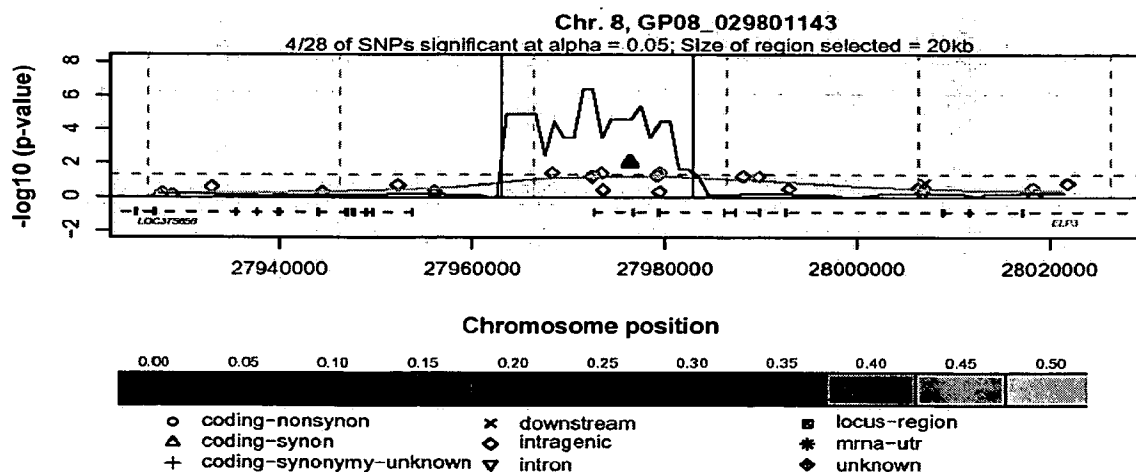


FIGURE 1C

CHDC1 – DISCOVERY P-VALUES (female only)

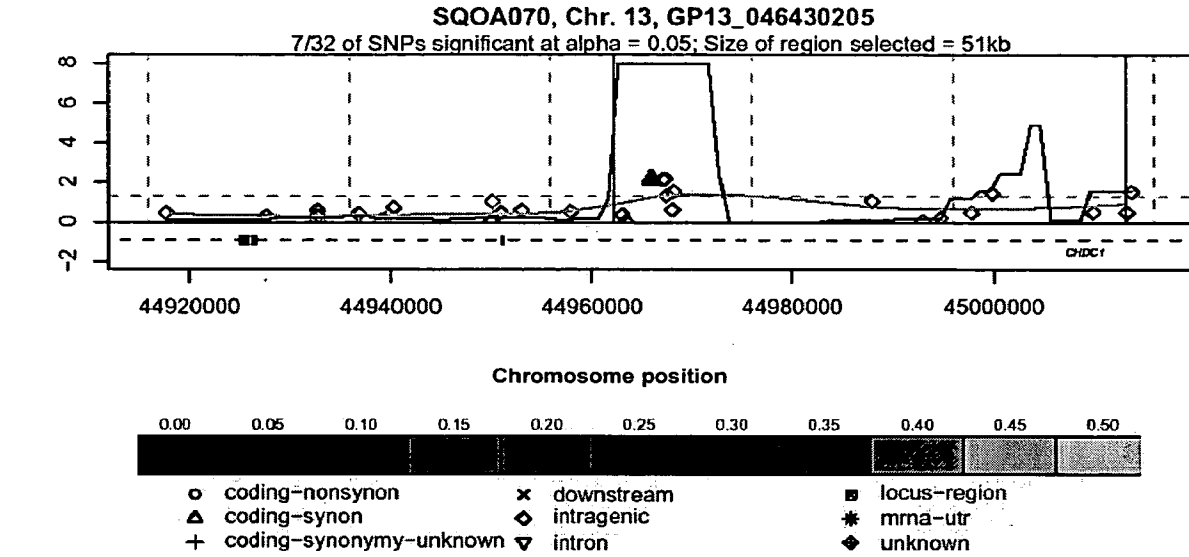
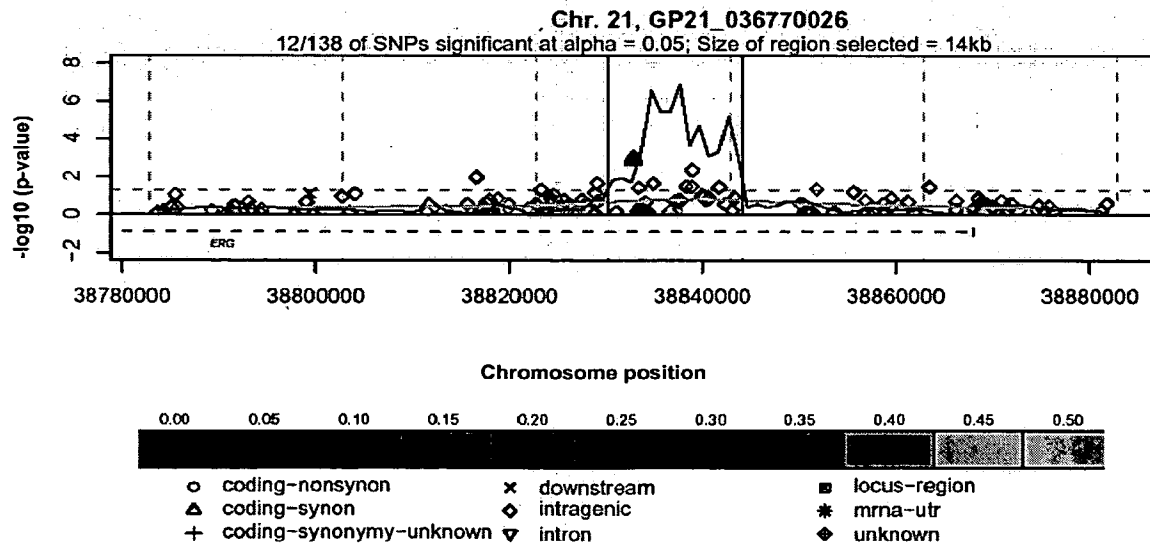


FIGURE 1D

ERG – DISCOVERY P-VALUES (female only)



## **Application Data Sheet**

### **Application Information**

Application Type::	Provisional
Subject Matter::	Utility
Suggested Group Art Unit::	Not Yet Assigned
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	METHODS FOR IDENTIFYING RISK OF OSTEOARTHRITIS AND TREATMENTS THEREOF
Attorney Docket Number::	524593008700
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets?::	2
Small Entity?::	Yes
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

### **Applicant Information**

Applicant Authority Type::	Inventor
Primary Citizenship Country::	US
Status::	Full Capacity
Given Name::	Steven
Family Name::	MAH
City of Residence::	San Diego
State or Province of Residence::	CA
Country of Residence::	US
Street of mailing address::	12820 Via Nieve #74
City of mailing address::	San Diego
State or Province of mailing address::	CA

Postal or Zip Code of mailing address:: 92130

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: Germany  
Status:: Full Capacity  
Given Name:: Andreas  
Family Name:: BRAUN  
City of Residence:: San Diego  
State or Province of Residence:: CA  
Country of Residence:: US  
Street of mailing address:: 3935 Lago Di Grata Circle  
City of mailing address:: San Diego  
State or Province of mailing address:: CA  
Postal or Zip Code of mailing address:: 92130

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: Germany  
Status:: Full Capacity  
Given Name:: Stefan  
Middle Name:: M.  
Family Name:: KAMMERER  
City of Residence:: San Diego  
State or Province of Residence:: CA  
Country of Residence:: US  
Street of mailing address:: 3825 Elijah Court, Unit 334  
City of mailing address:: San Diego  
State or Province of mailing address:: CA  
Postal or Zip Code of mailing address:: 92130

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: US  
Status:: Full Capacity

Given Name:: Matthew  
Middle Name:: Roberts  
Family Name:: NELSON  
City of Residence:: San Marcos  
State or Province of Residence:: CA  
Country of Residence:: US  
Street of mailing address:: 1250 Calle Prospero  
City of mailing address:: San Marcos  
State or Province of mailing address:: CA  
Postal or Zip Code of mailing address:: 92069

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: Sweden  
Status:: Full Capacity  
Given Name:: Rikard  
Middle Name:: Henry  
Family Name:: RENELAND  
City of Residence:: San Diego  
State or Province of Residence:: CA  
Country of Residence:: US  
Street of mailing address:: 7555 Charmant Drive, #1114  
City of mailing address:: San Diego  
State or Province of mailing address:: CA  
Postal or Zip Code of mailing address:: 92122

Applicant Authority Type:: Inventor  
Primary Citizenship Country:: United Kingdom  
Status:: Full Capacity  
Given Name:: Maria  
Middle Name:: L.  
Family Name:: LANGDOWN  
City of Residence:: San Diego

State or Province of Residence:: CA  
Country of Residence:: US  
Street of mailing address:: 3701 Yosemite Street  
City of mailing address:: San Diego  
State or Province of mailing address:: CA  
Postal or Zip Code of mailing address:: 92109

**Correspondence Information**

Correspondence Customer Number:: 25225

**Representative Information**

Representative Customer Number:: 25225